An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

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Dedicated to Anne, Aidan, Mom, Dad and Sars.

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Declaration

The work described in this thesis was performed in the Michael White Centre for Diabetes, Endocrinology and Metabolism, Hull Royal Infirmary, Kingston upon Hull between 2008 and 2010. All studies were jointly conceived, designed and analysed by the thesis supervisors and the author in person. The author was solely responsible for the collection of all blood samples and the setting up of the continuous glucose monitoring devices. Local ethical committee and audit department approval for the studies were obtained where appropriate.

Credit must go to the following people for their contribution to the studies in this thesis:-

Thesis supervisors, Professor Eric Kilpatrick, Honorary Professor of Clinical Biochemistry, Hull Royal Infirmary, Hull and Professor Stephen Atkin, Professor of Diabetes, Endocrinology and Metabolism, Hull York Medical School, Hull who assisted in the design and data analysis of all studies in the thesis and in the production of the final manuscripts for publication.

Ms Heidi Cox, Senior Clinical Biochemist, and Neill Lewis, Clinical Biochemist, Hull Royal Infirmary, who assisted with the data extraction and analysis of all glycated albumin samples. Ms H Cox was also responsible for producing the cross sectional population data evaluating glycated albumin and glycated haemoglobin.

Summary:

Diabetes Mellitus (DM) is a complex and challenging disorder for the health care provider. Delayed detection and poor glycaemic control are both associated with a significant increase in the risk of patient morbidity and mortality. With almost 1 in 25 people in the general population suffering from DM, it is crucial that this condition is managed and treated appropriately.

This thesis critically evaluates several factors that exert an influence on glycaemic control and glucose assessment in DM today. A significant proportion of the thesis discusses issues surrounding glycated haemoglobin (HbA1c) measurements in clinical practice. The thesis also examines several areas that influence glycaemic control in patients and evaluates other methods of glucose measurements including continuous glucose monitoring (CGMS), capillary glucose readings and glycated albumin.

Glycaemic control in patients with diabetes can be influenced by changes in lifestyle, diet and psychological stress. The first study in this thesis summarises the effect of a flooding disaster on the glycaemic control of patients with DM. Glycaemic control in this study was measured via longitudinal HbA1c measurements over a 24 month period. This was the first study in the UK to show that severe disruption to lifestyle, environment and psychological stress caused by a flooding disaster results in the worsening of glycaemic control in patients compared to the preceding months. The effect of the flooding was most pronounced in patients on insulin treatment (HbA1c 8.6% (8.3, 8.9) affected vs. 8.2% (8.1, 8.3) unaffected, ($p = 0.002$)).

Glycated haemoglobin (HbA1c) concentrations have been known to be unreliable and difficult to interpret when red cell physiology is abnormal. When this occurs, glycated albumin (GA) has been suggested as a better indicator to glycaemic control. This is evaluated in the next 2 studies in a group of patients with chronic kidney disease (CKD) and DM who received intravenous iron and erythropoietin stimulating agent (ESA) therapy. Both these drugs resulted in a significant fall in HbA1c levels (0.5% in iron, $p<0.01$, 0.6% in ESA, $p<0.01$). In contrast, GA values remained constant

(17.8% vs. 17.7%, p=0.59 in iron, 16.7% vs. 16.9%, p =0.71 in ESA). Glycaemic control was also stable throughout the entire 4 month study period which was evidenced by averaging ~1500 glucose readings in each patient. Glycaemic control in this study was monitored using continuous glucose monitoring devices (CGMS) and thrice weekly 7 point daily capillary glucose measurements.

Previous cross sectional studies have shown GA values to have a negative correlation with body mass index (BMI) such that more obese patients appear to have lower GA concentrations than those with lower BMI at the same level of glycaemia. However, little is known about the effect that significant weight loss could have on GA values in the similar individual. The next study attempted to evaluate any correlation between BMI and GA in group of patients with DM undergoing bariatric surgery. However, the study did not show any definite correlation between BMI and GA beyond that expected from their fall in HbA1c and mean glucose despite significant differences to BMI measurements pre and post surgery (mean±SD; 51.5±6.3 vs. 36.3±5.4 kg/m2).

The final 2 studies look into the clinical aspects of using HbA1c and plasma glucose as screening methods in the diagnosis and identification of patients at risk of DM. The studies were based on the diagnostic thresholds produced nationally in the UK by the Department of Health and the Joint British Society Guidelines (2005). The first of these studies show that using HbA1c rather than plasma glucose measurements would result in almost one fifth of patients who would have been previously diagnosed with DM being reclassified as ‘non diabetic and low risk’. In the second study, 21.1% of patients found to have impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) within primary care had not been further assessed in the subsequent 2 ½ years.

When treating patients with DM, it is absolutely crucial that clinicians are aware of the factors that can cause fluctuations in glucose control and interfere with glycaemic measurements. This can aid the clinician in tailoring the most appropriate therapy to suit each clinical scenario. This thesis demonstrates that glycaemic control and its measurements in patients with DM can be influenced by a variety of factors. Though

numerous assessment methods of glycaemic control exist, at the present time none of them are universally applicable to every patient and cannot be a sole substitute to clinical judgement.

Format of the thesis

This thesis evaluates several areas surrounding glycaemic control and its measurements in diabetes mellitus. The first chapter is the "Introduction" in which a literature review is carried out and the background for the studies performed for this thesis is set. Chapters 2 – 5 describe the 5 studies carried out for this thesis. Chapter 6 summarises the author's conclusions and discussion of the findings.

Aim of this work

1. To study the effect of stress and lifestyle disruption caused by a flooding disaster on glycaemic control in patients with DM as expressed by glycated haemoglobin (HbA1c)
2. To evaluate the effects of iron and erythropoietin stimulating agent therapy (ESA) on HbA1c, glycated albumin, mean blood glucose and continuous blood glucose monitoring.
3. To evaluate any correlation between body mass index (BMI) and glycated albumin (GA) values in patients undergoing bariatric surgery
4. To evaluate the utility of HbA1c and plasma glucose measurements in the diagnosis and screening of patients at risk of diabetes mellitus (DM).

Publications:

Some of the chapters in this thesis have been already accepted for publication or already published in peer reviewed journals. There are listed below in reverse chronological order:

JM Ng, M Cooke, S Bhandari, SL Atkin, ES Kilpatrick, ' The effect of iron and erythropoietin treatment on the Hb1c of patients with diabetes mellitus and chronic kidney disease' Diabetes Care 33:2310-2313

JM Ng, AJ Dawson, SL Atkin, ES Kilpatrick, 'New Recommendations in Diagnosis of Diabetes Mellitus from the Department of Health: Comparing the old and new.' Diabetic Medicine 27:244-245

JM Ng, SL Atkin, ES Kilpatrick (2009) 'Impaired Fasting Glucose and Impaired Glucose Tolerance: Follow up rates over 2 years within a Primary Care setting' Diabetic Medicine 27:123-123

Chapter 1:

Part 1: Diabetes Mellitus:

1.1.1 An introduction

The scope of the first chapter outlines the background of the thesis and its studies. The initial stage of the chapter relates to the diagnosis and assessment of glycaemic control with discussion on several issues surrounding glycated haemoglobin (HbA1c) and plasma glucose measurements. This is followed by discussion on some of the health risks associated with chronic hyperglycaemia and an evaluation of factors that can result in greater fluctuations of glycaemic control. The final part of the chapter addresses some other forms of glycaemic assessment including capillary glucose measurements, continuous glucose monitoring devices (CGMS) and glycated albumin.

1.1.2 Diabetes Mellitus: The scale of the problem

'Diabetes' was given its name by the Greek Physician Aretaeus of Cappadocia, and his contemporary, Galen of Pergamum, in the second century AD. It was during this time when he first recorded a disease with the symptoms of constant thirst (polydipsia), excessive urination (polyuria), loss of weight and a short life span. He named the condition 'diabetes', meaning 'a flowing through' to describe the phenomenon seen in individuals suffering from the condition where fluid appeared to pass right through the body (1).

Today, diabetes mellitus (DM) is a growing world wide disease which has reached epidemic proportions. The World Health Organisation (WHO) estimates that 180 million people in the world now have DM and this number is set to double by the year 2030. The prevalence of DM worldwide was estimated at 2.8% in 2000 and this is expected to rise to 4.4% by 2030 (2).

In the UK, the prevalence of DM has shown similar trends, with an increase from 2.8% in 1996 to 4.3% in 2005, in the population aged between 10-79 years. (2; 3)

More worryingly, this number is set to increase to 6.7% involving 2.6 million people, by the year 2010. Mostly, this rise has been attributed to a much more sedentary lifestyle in parallel with a marked increase in the incidence of obesity in the population.

Health expenditure in the management of DM is estimated to constitute up to 10% of all healthcare costs in the western world. The major part of this cost is in the treatment of the complications of DM. In the UK, the National Health Service (NHS) spends an estimated £500 million per annum on DM and its related complications. Prescription costs for this condition alone constitute the highest single cost in the entire NHS prescription budget (4).

1.1.3 Hyperglycaemic states:

Diabetes Mellitus (DM) is a term now used to describe a group of metabolic disorders characterised by resistance to the action of insulin, insufficient insulin secretion, or both (5). The manifestation of this condition presents itself as hyperglycaemia though concurrent disturbances in lipid and protein metabolism commonly coexist. As a result of this, the hallmark to the diagnosis of DM is through the biochemical verification of a hyperglycaemic state.

Patients with DM are classified into one of four broad categories, the first two being the most common; Type 1 DM (T1DM), Type 2 DM (T2DM), gestational DM (GDM) and DM due to other rarer causes. The first 2 of these diabetes subtypes are discussed and addressed in the scope of this thesis.

1.1.3.1 Type 1 DM (T1DM)

T1DM results as a consequence of the destruction of ß cells in the pancreas leading to absolute insulin deficiency. It presents mostly in the younger population although it can occur at any stage of life (6). The commonest cause of this is due to the autoimmune destruction of pancreatic ß cells though sometimes no obvious cause is found. T1DM accounts for 5-10% of those with diabetes (7).

The onset of this category of DM is variable although the majority of individuals suffering from T1DM are often symptomatic and present with weight loss, thirst, polyuria, polydipsia and diabetic ketoacidosis. Other more indolent adult-onset variants of T1DM exist but these are rare. Patients who develop this variant can be asymptomatic and often maintain sufficient insulin secretion for several years. Initial presentation is often only with modest fasting hyperglycaemia. Ketoacidosis and hyperglycaemic symptoms can develop but usually following a bout of severe infection or stress years later (7).

Despite the different ways T1DM may present clinically, its underlying pathogenesis inevitably leads to complete pancreatic ß cell destruction and eventually all patients with T1DM will require insulin therapy to maintain glycaemic control (6). Patients with T1DM are rarely obese and are prone to other autoimmune conditions such as Grave's or Addisons disease (7).

The diagnosis of T1DM is usually straightforward. Most patients will present showing classical signs of ketosis, extreme elevations of glucose and symptoms of hyperglycaemia. Because of this, timed or challenged glucose tests are rarely necessary and the diagnosis of this condition can often be made clinically (8). When the diagnosis is uncertain, other tests such as the measurement of C peptide levels or markers of immune destruction such as islet cell antibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD) and autoantibodies to tyrosine phosphatases IA-1 and IA-2 can be helpful in confirming the diagnosis (7).

1.1.3.2 Type 2 DM (T2DM)

T2DM is caused by a relative insulin secretory defect, insulin resistance, or a combination of both conditions (8, 9). Though numerous causes of this form of DM exist, autoimmune destruction of pancreatic ß cells does not occur. Patients may have normal or elevated insulin levels despite a higher than expected blood glucose level. T2DM is the commonest form of DM and accounts for 90-95% of all diabetic subtypes.

Most individuals with T2DM have abdominal visceral adiposity, a condition which is closely linked to insulin resistance (9). Patients who are not obese by traditional weight criteria may still have an increased percentage of body fat distributed over the abdominal region (7). Furthermore, patients with T2DM tend to exhibit a clustering of other health risk factors namely, hypertension, low high density lipoproteins (HDL) and high triglyceride levels. This condition is commonly referred to as the metabolic syndrome and is associated with an increased risk in overall mortality (9). Ketoacidosis seldom occurs in this form of diabetes and when it does present, tends to arise in association with other physiological insults such as infection (7).

Patients presenting with T2DM do not initially require insulin therapy and their condition can be managed by lifestyle intervention such as weight reduction or dietary control and oral hypoglycaemic agents (10). However, over time, there is progressive ß cell hyperplasia as a result of excessive insulin production which inevitably leads to progressive to ß cell failure and insulin deficiency. (11)

In T2DM, hyperglycaemia tends to develop very gradually. In the early stages, patients are often asymptomatic as the hyperglycaemic levels are not severe enough to produce symptoms (7). Because of this, the condition can remain undetected for many years and patients often present with established macrovascular and microvascular complications upon their initial diagnosis of T2DM (12).

Chapter 1:

Part 2: The rising tide glycaemia: Challenges in establishing the thresholds in the diagnosis of DM

1.2.1 Introduction:

The initial step in order to assess a 'hyperglycaemic state' is to first define the thresholds between what should be considered 'normal glucose levels' and 'hyperglycaemia'. This definition should in essence, identify individuals who are relatively 'free' from the risks of hyperglycaemia and have 'typical' ranges in blood sugar levels as compared to those who are at a greater health risk as a result of 'high' glucose levels.

1.2.2 The road to identifying a hyperglycaemia state:

An important component in the diagnosis of DM is the need for an agreed unified system of classification that can provide both a framework of identification and differentiation of its different forms and stages. This would allow for international collaboration towards improved epidemiological and clinical research, treatment and management of this worldwide epidemic.

The first widely accepted system of classification was established by the National Diabetes Data Group (NDDG) in 1979 (13) and was endorsed soon after by the World Health Organisation Expert Committee on Diabetes in 1980 (14). This classification was a significant step forward in the diagnosis and classification of diabetes mellitus. Under this system, the diagnosis of DM was to be based on plasma glucose measurements either as a timed (fasting) sample or challenged (following a 75g glucose load). T1DM and T2DM were both recognised as the two distinct and major forms of DM present. The NDDG also acknowledged DM as an etiologically and clinically heterogeneous group of disorders where hyperglycaemia was a shared biochemical manifestation.

Based on the NDDG/WHO guidelines, the diagnosis for DM was made when 1) there were classic symptoms of hyperglycaemia (thirst, polyuria, polydipsia) present; 2) a fasting plasma glucose (FPG) of ≥ 7.8 mmol/L or 3) plasma glucose 2 hours following a 75 g OGTT (2HPG), the of ≥ 11.1 mmol/L.

In selecting the diagnostic glucose value, the committee openly acknowledged that 'an arbitrary decision has been made as to what levels justifies the diagnosis of diabetes' which was based on the facts known of diabetes at that point. It was anticipated at that time that as knowledge of diabetes increased, further revision would be made in the future (13; 15).

In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ECDM) re-evaluated the thresholds and categorisation of glycaemia. In their revised consensus report, one of the significant changes made was to lower the diagnostic threshold for DM using FPG from 7.8 to 7.0 mmol/L (16). This was to improve the sensitivity for diagnosing DM using FPG making it similar to the existing 2HPG of 11.1 mmol/L. Furthermore, emerging evidence appeared to show that the risk for developing microvascular complications was closer to 7.0 mmol/L versus 7.8 mmol/L. This statement was later endorsed by the WHO in 1999 (17).

Until the mid 2009, nearly every method of diagnosis and classification of DM relied on the measurement of plasma (serum or blood) glucose concentrations in timed samples (e.g. FPG), random or following a metabolic stress test such of the 75g oral glucose tolerance test (8). Though there has been significant discussion regarding the use of HbA1c as an alternative diagnostic method before this time, it was never adopted as a viable method due to significant concerns regarding lack of standardisation and precision (see *Section 1.3.2 Issues surrounding HbA1c in the diagnosis of diabetes mellitus*) (5; 16; 18-20).

However, in 2009, glycated haemoglobin (HbA1c) was proposed as the preferred method to diagnose patients with DM an the International Expert Committee (IEC) (members appointed by the American Diabetes Association (ADA), European Association for the study of Diabetes (EASD), and the International Diabetes

Federation) (8). A cutoff HbA1c of ≥ 6.5% was put forward as the threshold for diagnosis of T2DM (8). Under these new recommendations, the thresholds for FPG and 2HPG were to remain unchanged but its measurement should be limited to circumstances where 'HbA1c measurements were unavailable' or when HbA1c values were known to be unreliable or inaccurate.

In the UK, this diagnostic recommendation using HbA1c was also recommended by the Department of Health (DoH). In the document 'Vascular Health Checks' published in 2009 (18), HbA1c was advocated in parallel with the use of FPG and 2HPG as a method of screening for patients with DM. The DoH proposed a similar HbA1c cutoff of 6.5% as the diagnostic threshold for the confirmation of DM.

In response to the IEC statement, the ADA published its own statement in the beginning of 2010 (7). The ADA reaffirmed the use of HbA1c in diagnosing DM but also recommended the continued measurements of both FPG and 2HPG as viable alternative methods. The HbA1c thresholds upheld by the ADA were unchanged from that proposed by the IEC statement. The issues regarding the limitations and disputes in the HbA1c measurements are discussed in *'Section 1.3.2: Issues surrounding HbA1c in the diagnosis of diabetes mellitus.'*

The current thresholds for the diagnosis of DM are described in Table 1.

Table 1: Glycaemic thresholds to the diagnosis of DM

Diabetes Mellitus	Result:
Fasting plasma glucose	≥7 mmol/L *
	or
2 hour plasma glucose ingestion of 75g of oral glucose load. (OGTT)	≥ 11.1 mmol/L *
HbA1c result	≥ 6.5% *

* Based on 2 sample readings unless symptoms present. Adapted from (8)

1.2.3 Patients at Risk of Future DM and Prediabetic States

Prediabetic states of both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (collectively known as impaired glucose regulation (IGR)) are biochemical diagnoses characterised by abnormal fasting and/or post load plasma glucose but at levels below what is expected for the diagnosis of DM. They are not considered clinical entities in their own right, but as health risk categories that confer an increased risk for the future development of DM and cardiovascular disease (19).

The term impaired glucose tolerance or IGT was first introduced by the NDDG/WHO synonymously in their statement in the diagnosis of DM in 1979/1980. It was defined using the threshold of 1) FPG < 7.8mmol/L and 2) 2HPG between 7.8-11.1 mmol/L. The IGT range was established in the light of increasing evidence showing an increased risk for developing macrovascular disease at glucose values even lower than that proposed for DM at that time (15). IGT was proposed to reflect a prediabetic state where individuals were at 'greater risk in progressing to DM but had a low frequency of diabetic symptoms, moderately increased risk of macrovascular disease and a rarity of "clinically significant microvascular disease".'

The prediabetes state of impaired fasting glucose (IFG) was only introduced almost 2 decades later along with the reclassification of DM by the ECDM/WHO in 1997. Impaired fasting glucose was defined as a FPG between 6.1-6.9 mmol/L inclusive. The purpose of the IFG was to describe a metabolic state between normoglycaemia and diabetes. In late 2003, a follow up report by the ADA recommended that the thresholds for IFG be lowered further to include a range of FPG $\geq$ 5.6 mmol/L and < 7.0 mmol/L (20). This was motivated by the need to optimise sensitivity and specificity of predicting future diabetes and in an attempt to make the number of patients diagnosed with IFG more comparable to the IGT thresholds (8). The WHO and many other diabetes organisations have not changed their thresholds for the categorisation of IFG (21).

In July 2009, along with its recommendations to use HbA1c as the method of diagnosis of DM over plasma glucose measurements, the IEC proposed to abandon the use of the terminology IFG and IGT (8). The reason given was that IFG and IGT 'failed to capture the continuum of risk' and these terms were to be replaced over time by a HbA1c value of 6.0-6.4% to identify patients at high risk of DM (8).

The ADA acknowledged the HbA1c as a viable measurement to define patients at risk of future DM but it maintained that the IFG/IGT thresholds were still valid in clinical practice (8). The ADA further revised the categorisation of patients at risk of DM using a lower threshold of HbA1c result from 5.7%-6.4% rather than the previous 6.0% of the IEC (10). It proceeded to stress that clinicians should be 'particularly vigilant' in patients with a HbA1c > 6.0% as this group is at very high risk of future DM.

In the United Kingdom, the Department of Health in their NHS Vascular Health Check Programme (18) maintained the terminology of both IFG and IGT and proposed a HbA1c between 6.0-6.4% in identifying individuals at risk. The term 'non diabetic hyperglycaemia' was used to describe these at risk group.

There is strong evidence showing that IGR and high normal HbA1c values confer a significant increase in the risk of future DM. A study in Mauritius over 5 years found that proportion who progressed to DM over the study period was 38.1% in those with both IFG and IGT, 20.8% in those with isolated IGT and 21.6% in isolated IFG (22). Similar results have been seen with using HbA1c measurements. Prospective studies have shown that patients with an HbA1c over the range of 5.5-6.0% have a 5-year cumulative incidence of diabetes of between 12 to 25% (23-25). A study by Selvin et al (26) on more than 11,000 patients reported a hazard ratio of 1.86 (1.67,2.08) of developing DM and 4.48 (3.92,5.13) for the HbA1c range of 5.5-5.99% and 6.0-6.49% respectively using the HbA1c range of 5.0-5.49% as a reference. The authors of the study found comparable prognostic values of HbA1c and FPG for identifying patients at risk of DM. The National Health and Nutrition Examination Survey

(NHANES) reported the HbA1c value that corresponded best with IGR was between 5.5-6.0% and that the FPG of 6.1 mmol/L best correlated to a HbA1c value of 5.6% (7).

The identification of patients with prediabetic states and IGR can help the early commencement of therapy and enable future screening. However, the risk of developing DM using any measure or index of glycaemic control appears to be a increasing continuum, extending well into the normal ranges (7; 8). A study performed in Israel found that the risk of developing DM was increased even when assessing men with a FPG < 4.5 mmol/L versus those with FPG ≥ 4.8mmol/L (27). Therefore, in the absence of a specific lower threshold defining when intervention should be implemented, a lower glycaemic limit will always be somewhat arbitrary and needs to take into consideration the potential limit to resources available.

The current glucose values for the diagnosis of these categories are described in Table 2:

Table 2: Glycaemic thresholds to the diagnosis of impaired glucose regulation and patients at risk of developing future DM

Impaired fasting glucose	Plasma glucose
WHO and ECDM criterion (19; 31)	6.1- 6.9 mmol/L
ADA criterion 2003 (28)	5.5 – 6.9 mmol/L

Impaired glucose tolerance	Plasma glucose result following 75g OGTT:
Unified criterion (21; 29)	7.8- 11.0 mmol/L

High risk for future DM	HbA1c values
IEC and DoH criterion (8; 18)	6.1- 6.9 %
ADA criterion (10)	5.5 – 6.9 %

Chapter 1:

Part 3: HbA1c in the measurement and detection of hyperglycaemic states:

1.3.1 Introduction & History of HbA1c:

It would be near impossible to discuss the evaluation of glycaemic control in DM without referring to glycated haemoglobin (HbA1c). At the present time, HbA1c is the most commonly measured method of assessing chronic glycaemia in clinical practice (30). It has been used as a surrogate marker of long term glycaemic control in patients with DM for more than 30 years, both in clinical practice and in countless studies and trials (31).

Glycation of haemoglobin occurs following exposure to glucose involving a 2 stage process within the erythrocyte. The first follows a transient rise in glucose leading to a reversible formation of an aldimine. Following prolonged exposure, an Amadori rearrangement takes place forming an irreversible ketoamine. This effect is permanent until the destruction of the haemoglobin (32).

The value of the HbA1c measurement is dependent on the amount of circulating glucose and that of haemoglobin. The value of the HbA1c (which are expressed as a

percentage of total haemoglobin) gives a time-weighted indication of the average glucose over the lifespan of the red cell (33).

HbA1c was first described in late 1960s, when Rahbar and his colleagues from the University of Tehran discovered a 'diabetic haemoglobin component' on the electrophoresis of 2 patients with DM (34; 35). This component was later found to be identical to the HbA1c. The clinical utility of HbA1c as a measurement of glycaemic control was initially proposed by Trivelli and colleagues in 1971 who suggested a possible relationship between mean blood glucose, long term diabetic complications and HbA1c values (36). This theory was later supported by numerous studies showing that the increased proportions of HbA1c in DM could reliably measure the glycaemic control over the preceding 6-8 weeks (37; 38). Around 1977, the HbA1c was first introduced to clinical laboratories for diabetes monitoring.

At the present time, the HbA1c is used worldwide as the marker of long term glycaemic control and also a therapeutic target in the prevention and delay of the development of hyperglycaemic complications (20, 23, 24). More recently, the HbA1c has also been proposed as a diagnostic tool for DM and as a screening test in identifying patients at future risk of developing the condition (7). This is discussed in *'Section 1.3.2 Issues surrounding HbA1c in the diagnosis of diabetes mellitus.'*

1.3.2 Issues surrounding HbA1c in the diagnosis of diabetes mellitus

Years before the International Expert Committee (IEC) consensus statement in 2009, measuring HbA1c to diagnose DM in patients had been debated. Several early studies in the late 1970s (39; 40) first pursued this possibility but initial findings were hindered by inconsistent results due to the different glucose cut offs used in the OGTT during that time. Following this, other concerns were raised as to whether the HbA1c

had sufficient specificity or sensitivity to the detect patients with mild hyperglycaemia (41). Equally concerning were the lack of standardisation in HbA1c measurements and its uncertain correlation to glucose measurements (42). Since then, numerous studies and reviews have continued to debate the issues surrounding HbA1c as a diagnostic marker (5; 16; 18-20).

At first glance, the HbA1c holds several distinct advantages as a diagnostic method of DM over the conventionally measured plasma glucose. The ability to use a similar test to diagnose both DM and evaluate the results of therapy is an attractive clinical prospect in comparison to using two different tests; one to diagnose DM and then another to monitor glycaemic control.

From a practical standpoint, the sampling of HbA1c is easier as it can be drawn at any time as opposed to a fasting state (43). Furthermore, it represents the glycaemic state of an individual over a 1-3 month period and is constant over periods of acute fluctuations in glucose levels (i.e. illnesses or stress). In comparison, accurate plasma glucose measurement requires fasting or a 75g OGTT and can also be subject to acute fluctuations. This can be inconvenient for both the patient and the healthcare provider (8). Additionally, the ease of measuring HbA1c allows for 'opportunistic' screening of patients as they do not require another clinic visit for a blood test (10).

The diagnostic thresholds for DM are currently based on the risks of adverse clinical outcomes, particularly diabetic retinopathy (16). Using HbA1c measurements based on this principle appears attractive as observational studies have shown consistent correlations between HbA1c and microvascular disease (44; 45). Furthermore, a longitudinal study by Tapp et al. showed that HbA1c was a greater predictor of retinopathy than FPG (45).

HbA1c samples are relatively more stable following collection. This attribute results in less pre analytical instability when compared to plasma glucose measurements (46). A study by Miller et al. (47) of laboratory instruments used to measure plasma glucose revealed significant bias from the reference method. Also, glucose values

have been known to decrease by 4.6% at 2 hours and 7% after 24 hours of storage despite the use of sodium fluoride tubes to inhibit in vitro glycolysis (48-50).

Intrasubject variations of HbA1c values are also more stable than that of plasma glucose measurements. The biological variability of HbA1c within an individual is smaller than that of FPG and significantly more so than 2 hour post 75g OGTT plasma glucose (2HPG) (coefficient of variation 3.6% vs. 5.7% vs. 16.6% respectively) (51). In other words, repeat sample measurements would give more consistent results using HbA1c and lead to a lower risk of an individual being misclassified as having a normal/abnormal glucose metabolism.

When HbA1c was first introduced to clinical practice, one of the main problems arose from the significant range of different values derived from a single sample when it was performed in different laboratories. The main issue with this was due to the lack of standardisation in HbA1c measurements at that time (42). The initial HbA1c assays also lacked precision, predominantly due to inconsistent calibrators or materials for quality control purposes (52). There have been significant changes that have taken place since that time. Most of this followed the DCCT study results published in 1993 which renewed efforts to establish a globally standardised HbA1c measurement (53). The encouraging results showing the benefits of tight glycaemic control were amongst the significant driving forces in harmonising the majority of the HbA1c assays in laboratories worldwide to that of the DCCT.

However, these initial assays were harmonised based on reference methods that measured a mixture of glycated haemoglobins (30). Since 2007, there has been an internationally agreed consensus for HbA1c values to be reported using the International Federation of Clinical Chemistry (IFCC) units (mmol/mol) via an agreed standardised methodology (54). The new IFCC measurement was developed to a new reference method that specifically measured the concentration of one molecular species of HbA1c. It is hoped that all HbA1c values in the future would be globally expressed using this new IFCC measurements.

1.3.3 Issues surrounding HbA1c as a surrogate marker of glycaemic control and mean blood glucose

Despite the near worldwide reporting of HbA1c as a measurement of chronic glycaemia, it is by no means a perfect test. Amongst the limitations in HbA1c is that its value is inherently reliant on red cell physiology and structure. Abnormalities to this can occur in patients with a variety of clinical conditions including renal failure, uraemia, iron deficiency and haemolysis.

In iron deficient patients, HbA1c levels can be 1.2% higher than iron replete controls with apparently similar glycaemic levels (55). In patients with anaemia, treatment with haematopoietic agents such as erythropoietin stimulating agent or iron therapy has been reported to cause a fall in HbA1c values (56). In uraemic patients, formation of carbamylated haemoglobin (the product of the non-enzymatic, covalent attachment of isocyanic acid (from the spontaneous dissociation product of urea) to haemoglobin) can result in the overestimation of HbA1c values via several assay methods (57; 58).

In parallel to this, individuals with similar HbA1c values have been shown to exhibit a wide variation in mean blood glucose ranges (MBG) (59). In other words, though a high HbA1c is associated with a general rise in overall glucose levels in any one individual, it does always not correlate to the same MBG value in every patient tested. For instance studies have found that a HbA1c value of 7.0% can correspond to a MBG range between 8 and 11 mmol/L in different subjects with T1DM (60) and 6.8-10.3 mmol/L in both T1DM and T2DM (61). This variation seen in HbA1c values and MBG has given rise to the theory of intersubject variation in haemoglobin glycation rates (high and low glycators) (59; 60). High glycators have a consistently higher HbA1c for an expected MBG whilst low glycators have the opposite; a low HbA1c for a predicted MBG. The difference seen in haemoglobin glycation rates are believed to be due to a difference in erythrocyte survival or genetic elements.

Concerns have also been raised regarding the data from some studies comparing plasma glucose and HbA1c with the risk of complications. Some show FPG and the 2HPG possessing a greater association with the risk of developing microvascular disease than HbA1c (62) and some the opposite (45). In fact, the position statement from the American Diabetes Association in 2010 concurred that there was no full concordance between HbA1c and glucose based tests (7).

This is illustrated in the same report when comparing plasma glucose and HbA1c in the diagnosis of DM. In this report, it was stated that 'the A1c cut off point of ≥ 6.5% identifies one-third fewer cases of undiagnosed diabetes than a fasting glucose cut off point of ≥ 126mg/dL (7.0 mmol/l).' In using the HbA1c cut off of ≥ 6.5% to screen patients for DM, there would be 1.6% of adult individuals who would have undiagnosed DM, 2.5% if using FPG and 4.9% if using 2HPG alone. The sole use of HbA1c has a sensitivity of detecting undiagnosed DM of only 42.8% (63).

The HbA1c remains a very useful test in the measurement of glycaemic control. Though not always reliable in every patient, it is by far the most universally accepted method of glycaemic measurement in clinical practice today. Nonetheless, it is important that clinicians are aware of the potential inaccuracies of the HbA1c measurements. Ultimately, the complications of DM most likely stem from the effects of chronic hyperglycaemia and therapy should therefore be tailored to treat this and not the HbA1c value alone.

Chapter 1:

Part 4: Examining the thresholds and glycaemia: mortality and morbidity

1.4.1 Retinopathy:

Diabetic retinopathy develops in a significant proportion of patients who have DM. It is a highly specific vascular complication of DM and is the most common cause of visual disability and legal blindness in developing countries amongst the age group of 20-74 years (64). The risks associated with the development of diabetic retinopathy in patients include the duration of diabetes (10), presence of nephropathy (65), hypertension (66) and chronic hyperglycaemia (67; 68).

The prevalence of retinopathy in patients with T2DM has been estimated to be approximately 20% at the time of diagnosis and this figure rises to 60% after 20 years (69). In the 25 year study of the Wisconsin Epidemiology Study of Diabetic Retinopathy (68), there was a progression of retinopathy in 83% of the 955 T1DM patients (of which 42% progressed to proliferative retinopathy) and a regression noted in 18% over a 25 year study period.

The current glycaemic thresholds of HbA1c of 6.5% and FPG of ≥126 mg/dL (≥7.0 mmol/L) to diagnose DM have been based on epidemiological studies that appear to indicate this point as the threshold in which the risk of retinopathy rises (7; 8; 16). Below these value, the prevalence of retinopathy appears low but consequently increases in a linear fashion at higher glycaemic levels.

Many studies including the Diabetes Control and Complications Trial (DCCT) (70) and United Kingdom Prospective Diabetes Study (UKPDS) (71) have evaluated the relationship between glycaemic control and retinopathy. The DCCT trial was a landmark study on 1441 patients with T1DM who were randomised to receive either intensive or conventional diabetes therapy over the mean study period of 6.5 ±1.6 years. From these study results, it was established early on that intensive blood sugar control was important in preventing retinopathy, microalbuminuria and neuropathy (70). The results of the study showed a significant risk reduction in developing

diabetic retinopathy (76% (CI 62-85) without retinopathy, 54% (CI 39-66) mild to moderate retinopathy and 47% (CI 14-67) proliferative and severe non proliferative retinopathy) between the intensively treated patients vs. conventionally treated patients (HbA1c 9.1% vs. 7.2%) over the study period. Follow up epidemiological analysis of this study established that the HbA1c was the strongest predictor to the progression of retinopathy (72). It was shown that a reduction of 10% in the percentage HbA1c (e.g. 8% to 7.2%) was associated with a greater than 40% decrease in the risk of retinopathy in both the intensively treated group and conventionally treated arms of the study. In addition to this, the beneficial effects on retinopathy seen initially appeared to persist after the end of the study 4 years later despite the glycaemic control in both conventional and intensively treated arms narrowing to a HbA1c of 8.2% and 7.9% (73).

The UKPDS study was a study of 3,867 newly diagnosed patients with T2DM randomised to either intensive (sulphonylurea or insulin, or in overweight patients, metformin) and conventionally treated (diet) arms (74). The intensive treatment FPG target was below 6 mmol/l and conventional treatment aimed to obtain the best fasting blood glucose as possible with drugs added only if symptoms occurred or FPG exceeded 15 mmol/l. The UKPDS reported 21% reduction in the risk of retinopathy between the intensively controlled group and the conventionally treated group (HbA1c 7.0% vs. 7.9%) over 10 years. The results of the benefits of intensively treated glycaemic levels on the risk of retinopathy concurred with the findings of the DCCT.

Though chronic hyperglycaemia has been shown to be strongly associated with an increased risk of developing diabetic retinopathy, there are conflicting results on the degree of glucose intolerance necessary to cause a significantly increased risk. An analysis of three cross sectional studies in Australia and the USA of nearly 12,000 patients in 2008 by Wong et al. found no consistent glycaemic thresholds for the presence or incidence of retinopathy across different population groups (44). The

study reported that a plasma glucose value of 7.0 mmol/L had a modest 10.2% sensitivity but 97.4% specificity and a positive predictive value of 3.9 to detect incident retinopathy. Furthermore, for the patients with a FPG ≤ 5.6 mmol/L, the incidence of retinopathy was as high as 7-13%. Equally concerning is that in the DCCT trial, about 10% of patients appeared to develop diabetic retinopathy despite good glycaemic control (mean HbA1c ≤6.87%) and more than 40% of patients with a mean HbA1c ≥ 9.49% did not develop this complication over the study period (75). The reasons for this is unclear but possible explanations for this include previous glycaemic exposure, genetic predisposition, differences in the BMI and variable intersubject glycaemic thresholds to the development of diabetic complications.

1.4.2 Diabetic nephropathy and chronic kidney disease:

Diabetic nephropathy can affect up to a third of patients with DM and is the leading cause of end stage renal disease (ESRD) in the world. About 40% of all patients requiring renal replacement therapy have DM (76). In 2001, it was estimated nearly 11,000 patients with DM in the UK were on long term renal replacement (77).

Persistently raised urinary albumin excretion (microalbuminuria defined as urinary albumin excretion of 30-299 mg/24 hours and macroalbuminuria > 300mg/24 hours) has been shown to be the earliest stage of diabetic nephropathy in patients with T1DM and a marker to the development of nephropathy in T2DM (10). Patients with DM and microalbuminuria suffer an accelerated decline in renal function with an annual fall in GFR of 5.3 mL/min as compared to 0.2 ml/min in normoalbuminuric patients (78).

The presence of either increased urinary albumin excretion or abnormal eGFR values (<60 mL/min/1.73 m^2) both confer an increased risk of mortality in patients (79). Though both of these measurements are used to detect renal dysfunction, studies have shown that abnormal GFR values and urinary albuminuria may not be simultaneously present in up to 40% of patients with DM and CKD (80; 81). There are several

explanations to possibly account for this. Firstly, pathologically proven diabetic nephropathy can exist in the absence of urinary albuminuria, though this finding is unusual (82; 83). Secondly, the cause of CKD in patients with DM is heterogeneous and renal dysfunction is often associated with other concurrent diseases other than diabetic nephropathy such as hypertension or reno-vascular disease (84). There are therefore a variety of ways in which vascular and tubulointerstitial morphology can be affected in patients with DM and this in turn gives rise to the abnormal variations in urine albumin excretion and GFR values encountered in clinical practice.

The ADA recommends the routine screening for diabetic nephropathy and CKD in patients with DM using both urine albumin excretion and serum creatinine measurements (10). A spot urine test for albumin creatinine ratio (ACR) measurements has been found to be reliable and is recommended as the initial test for microalbuminuria. Serum creatinine values are measured to calculate the estimated glomerular filtration rate (eGFR) and to stage the level of chronic kidney disease (CKD) if present. The most widely accepted methodology of staging CKD is by using the 2002 National Kidney Foundation classification (85) in the table 3 (in page 34).

Using the data from the UKPDS, Adler et al (86) showed that the yearly rate of progression from the initial diagnosis of DM to microalbuminuria was 2.0%, from microalbuminuria to macroalbuminuria 2.8%, and from macroalbuminuria to an elevated plasma creatinine concentration or the need for renal replacement therapy 2.3%. The ten year prevalence in patients with T2DM of progression to microalbuminuria was 24.9% and macroalbuminuria 5.3% (86). For patients with macroalbuminuria, the prevalence of developing an elevated plasma creatinine (>175mmol/L) or requiring renal replacement therapy (RRT) over the similar period was 0.8%.

Table 3

CKD stage	Description	eGFR (mL/min/1.73 m^2)
I*	Kidney damage with normal or increased GFR	≥ 90
II*	Kidney damage with mildly decreased GFR	60-89
III	Moderately decreased GFR	30-59
IV	Severely decreased GFR	15-29
V	Kidney failure	< 15 or dialysis

* kidney damage defined as abnormalities in urine measurements (e.g. microalbunuria), blood, pathologic or imaging tests.

Adapted from (85)

Microalbuminuria is an early marker to the increased risk of developing diabetic nephropathy, end stage renal disease (ESRD), diabetic retinopathy and cardiovascular disease (76; 86-88). Patients who subsequently develop macroalbuminuria are at even greater risk of mortality. The annual rate of cardiovascular mortality is reported to be 0.7% for patients with no nephropathy, 2.0% for microalbuminuria, 3.5% for macroalbumuria and 12.1% for patients with elevated creatinine (>175mmol/L) or renal replacement therapy (RRT) (86). Patients with elevated plasma creatinine or on RRT are at significant risk and have an annual death rate of 19.2%.

Tight glycaemic control has been shown to positively influence the risk outcomes in diabetic nephropathy. On a cellular level, it has been known to reverse glomerular hypertrophy and hyperfiltration, both important mechanisms for early glomerular injury (89). Clinically, this has been shown following the results of the Diabetes Control and Complications Trial (DCCT) (70) and the United Kingdom Prospective Diabetes Study (UKPDS) (74). Of the 1365 T1DM patients with normal albumin

secretion at baseline in the DCCT, patients randomised to receive intensive glycaemic therapy were found to have a reduction in new onset microalbuminuria (40mg/24 hours) of 39% and macroalbuminuria by 54% (> 300mg/24 hours) when compared to those receiving conventional treatment over the mean study period of 6.5 years (90). Similarly, the UKPDS reported similar benefits with an incidence of nephropathy of 19.2% in patients receiving intensive glycaemic therapy as compared to 25.4% in the conventionally treated arm (HbA1c 7.8% vs. 8.4% respectively).

In an observational study, Araki et al noted that lower values of HbA1c of < 6.95% were independently associated with regression and remission of microalbuminuria (defined as a 50% reduction in the urine albumin excretion rate) in patients over a 6 year period (91). A meta analysis of 226 patients with T1DM of seven trials found that the odds ratio for increasing albumin excretion was 0.34 in patients receiving intensive therapy as compared to conventional therapy (92).

In contrast, the evidence for intensive glycaemic therapy in improving progressive renal injury once macroalbuminuria or over proteinuria has developed is poor (93). The long term maintenance of normoglycaemia, however, may be of benefit. A small study of a group of T1DM patients who have achieved this following pancreatic transplants were found to have improvements to glomerular structure but only after 10 years (94). This therefore suggests that in the absence of restored normoglycaemia, (currently achievable only via pancreas or islet transplantation) there is of lack substantial benefit in overt diabetic nephropathy from strict glycaemic control alone and implies that other factors (such as intraglomerular hypertension and glomerular hypertrophy) are contributing to the progressive glomerular injury.

The early initiation of intensive glycaemic therapy to near-normal glucose levels appears to reduce the future onset or progression of diabetic nephropathy. This finding was reported in the EDIC study, where the T1DM patients from the formerly intensive treated arm from the DCCT 8 years post study were found to have sustained

reduction in odds to the development of microalbuminuria of 59% (95% CI 39-73) and macroalbuminuria of 84% (95% CI 67-92) as compared to the conventionally treated arm despite the fact that HbA1c values of both groups being relatively similar (95) not long after the end of the study period. An analogous finding was reported in the post study follow-up of the UKPDS. Despite an early loss of glycaemic difference between the intensively treated and conventionally treated T2DM patients, patients who previously had favourable glycaemic control continued to show continued benefits in the risk of nephropathy after 10 years following the end of the study (96).

1.4.3 Erythropoesis stimulating agent therapy (ESA) in the treatment of anaemia in chronic kidney disease (CKD) and diabetes mellitus.

Anaemia is a common feature of patients with CKD, particularly when glomerular filtration rates (GFRs) decline below 60 mL/min per 1.73 m2 (97). The prevalence of anaemia (defined as Hb <12 g/dL in men and <11 g/dL in women) increases from 1% at eGFR of 60 mL/min to 9% when eGFR is 30 mL/min and to 33-67% once eGFR falls below 15 mL/min (98). Patients with DM and CKD are at increased risk. Anaemia develops more frequently in this patient group (99; 100) and haemoglobin values are significantly lower as compared to other matched patients with CKD of the same eGFR who do not have DM (99; 101). Patients who develop anaemia have poorer quality of life (102; 103) and suffer from an acceleration of long term microvascular and macrovascular complications (104).

In an analysis of the Reduction in Endpoints in NIDDM with the Angiotension II Antagonist Losartan (RENAAL), anaemia was shown to be an independent predictor for the progression to ESRD in patients with T2DM and nephropathy (105). This effect is believed to be as a direct result of renal tissue hypoxia or a reduction in renal blood flow secondary to renal sympathetic activity.

In a cross sectional study in patients with DM, Qing and colleagues reported that severe retinopathy was 5 times more likely in patients with low haemoglobin values as compared to those with mild retinopathy (106). Similarly, the Early Treatment Diabetic Retinopathy Study (ETDRS) concluded a hazard ratio of 1.52 in anaemic patients with DM for developing proliferative retinopathy or visual loss (107).

There is also an increased risk of cardiovascular disease due to left sided cardiac failure (108) and increased cardiac related hospitalisation and death (102). Left ventricular hypertrophy as a result of maladaptive cardiac remodelling can result and is thought in part to be responsible for the increased risk of mortality (109).

Patients with DM and CKD can develop anaemia for a variety of reasons, though the most common cause is erythropoietin deficiency (110; 111). In an Australian study of 722 patients, researchers noted that at all levels of anaemia, patients with DM were more likely to have inappropriately low erythropoietin values as compared to normoglycaemic controls (112).

EPO is a glycoprotein produced by the peritubular cells of the kidney. Its primary function serves in promoting the proliferation and differentiation of erythroid progenitor cells in the bone marrow resulting in an increase in the production of red cells in the body (110). Stimuli such as high altitudes (where oxygen levels are low) and chronic bleeding can hence trigger the release of EPO. Chronic hyperglycaemia can accelerate the destruction of peritubular cells in the kidney and this in turn causes the low levels of circulating EPO and the its poor response to anaemia.

Though EPO deficiency is commonly implicated, anaemia in DM and CKD can also result from other causes including iron, B12 or folate deficiency and blood loss (occult or overt) (110). Functional or absolute iron deficiency frequently occurs and is also the most likely cause of treatment failure in patients on ESA therapy (113).

ESA therapy was first approved by the Federal Drug Administration (FDA) for the treatment of anaemia associated with chronic kidney disease in 1989 (114). The introduction of ESA therapy was a revolution to the treatment of this condition as a significant proportion of these patients frequently fail to respond to iron therapy and had to otherwise rely on regular blood transfusions to maintain haemoglobin levels (110; 113). This was further encouraged by a flurry of early studies which supported the clinical utility of maintaining a higher range of haemoglobin.

Treated patients with higher haemoglobin concentrations were reported to enjoy improvements in quality of life (109; 115), increased energy levels (109; 116), better exercise tolerance (109; 117), restored sexual function and improved appetite (109).

Unfortunately, results from subsequent randomised trials attempting to show benefits in using ESAs to raise haemoglobin concentrations to higher targets have somewhat surprisingly suggested the opposite. Studies on non DM individuals such as the Normal Haematocrit Study (118) were terminated prematurely, with study results showing an increased risk of cardiovascular mortality in treating patients to higher haemoglobin targets using ESA therapy. Other studies, including the Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) (115) failed to demonstrate harm nor benefit from the early treatment and complete correction of anaemia with CKD.

Study results of ESA therapy in anaemic patients with CKD and DM are no different. The recent TREAT study (119), a multi centred trial randomised more than 4000 anaemic patients with DM and CKD to receive either ESA therapy to achieve a haemoglobin value of 13 g/dL versus placebo. The trial found that ESA did not reduce any of its primary end points of death or cardiovascular events but was conversely associated with an increased risk of stroke.

The reason behind the lack of benefit of ESA therapy in these studies is unclear. Persistently high or brisk rises in haemoglobin concentrations or changes within systolic blood pressure following ESA therapy in these studies could all serve as possible contributory factors. For instance, increasing haemoglobin concentrations exceeding one g/dL per 2 week period have been shown to be associated with an increased risk of adverse cardiovascular events (114).

In 2006, the National Institute for Health and Clinical Excellence (NICE) produced guidelines recommending physicians to consider the use of ESA therapy in iron replete adults with CKD and haemoglobin (Hb) values less than or equal to 11 g/dL. Therapy was to be tailored to maintain a target haemoglobin between 10.5-12.5 g/dL (110). This recommendation was replicated by the National Kidney Foundation (NKF) Dialysis Outcomes Quality Initiative (DOQI) in 2007 which supported a haemoglobin target in the range of 11 to 12 g/dL in all patients with CKD but also stressed that target values should not exceed 13 g/dL (120).

1.4.4 Hyperglycaemia, macrovascular disease and mortality.

Patients with diabetes DM have a 2-4 times greater likelihood of developing cardiovascular disease (CVD) as compared to local population. Approximately two thirds of all deaths in patients with DM are as a result of cardiac disease or stroke (10).

There is a well documented relationship between rising glycaemic levels and the risk of macrovascular disease and mortality. The Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study of nearly 30,000 participants reported a strong association between FPG and 2HPG and patient mortality even at thresholds below that currently used for DM (121). Levitan et. al performed a meta-analysis of 38 prospective studies (122) and concurred that rising levels of glycaemia even in the non diabetic range were associated with an increased

risk of fatal and non fatal CVD. A linear relationship was proposed in post challenge glucose levels and a possible threshold of risk in FPG was reported at 5.5 mmol/L.

As with plasma glucose measurements, studies on HbA1c and mortality have unsurprisingly shown a similar pattern (123-125). Using data from the ARIC study, Selvin et al. reported that rising HbA1c's (including that at the non diabetic ranges) was associated with an increasing risk to all cause mortality using the HbA1c range of 5-5.4% as reference (123).

A meta-analysis of 13 prospective cohort studies (including the UKPDS) found that for every 1% increase in HbA1c there is an associated increase in the relative risk of any cardiovascular event of 1.18 (CI 1.10-1.26) in patients with T2DM and 1.15 (CI 0.92-1.43) in T1DM (126). Analogous to this, a prospective study of men in Norfolk cited a 28% greater risk of mortality independent of serum cholesterol, age, blood pressure, BMI and smoking with every 1% increase of HbA1c (127).

Despite robust evidence showing an association between rising glucose values with CV events and death, the ideal therapeutic glycaemic targets in risk reduction remains uncertain. At the present time, none of the studies that have been able to consistently show a set glycaemic threshold where the risk of mortality rises sharply.

The initial findings from the UKPDS and the DCCT failed to show the benefits of intensive glycaemia therapy in reducing the risk of cardiovascular disease (74). Though the findings of the UKPDS failed to reach statistical significance, (p=0.052) there was a suggestion of a potentially favourable trend in the benefits of tight glycaemic control. In the DCCT, the benefits of intensive treatment of glycaemic control in CVD were inconclusive due to the low incidence of CV episodes. This was because the study initially recruited patients between 13-39 years and only 6 patients in the study had suffered from non-fatal myocardial infarction, stroke or cardiovascular death when the first results were published.

The results of the ACCORD (128) and ADVANCE (129) trials published in 2008 added further concern over the effects of tight glycaemic control and CV mortality.

Both the ACCORD and ADVANCE were large, international, multicentred clinical trials involving T2DM patients aimed at studying the impact of intensive glycaemic therapy (targeting as near to normal blood glucose levels possible) on overall mortality (HbA1c targets being ≤6.0% in ACCORD and ≤6.5% in ADVANCE). Both these trials failed to show any beneficial effects of intensive glycaemic therapy on CV risk. Rather adversely, the ACCORD study appeared to show the opposite and was terminated 3 years prematurely due to an interim analysis which showed an excessive rate of CV deaths.

Follow up epidemiological studies of the DCCT and UKPDS provided more encouraging results. The follow-up study of the UKPDS (96) and the EDIC/DCCT study (130) found significantly fewer cases of macrovascular disease in the formerly intensive treated group versus the control group despite no significant difference in HbA1c levels early on after the end of both trials. The relative risk reduction was 15% for myocardial infarction and 13% for all cause mortality in the follow-up UKPDS results.

Despite a strong link between the hyperglycaemia and cardiovascular risk, current study data indicate that intensive glycaemic control is unlikely to lead to any significant improvements to macrovascular complication in the short term. However, it appears to be in favour of a better macrovascular outcome in the longer term especially in the early intensive glycaemic management of patients newly diagnosed with DM.

Chapter 1

Part 5: External influences to glycaemic control

1.5.1 The need for early assessment & follow up of patients at risk:

The screening of patients for DM is important. Patients who present with T2DM often have no symptoms of hyperglycaemia. Therefore, chronic hyperglycaemia can exist for an extended period before the diagnosis of DM is made (18). A 6 year epidemiological study of patients from the UKPDS found that 37% of patients diagnosed with DM had retinopathy at presentation and diagnosis (71). A study by Harris et al. estimated that the onset of T2DM could be up to 7 years before the actual clinical diagnosis (12).

In prediabetic states and patients at future risk of DM, studies have also shown that prompt intervention (either with medication or lifestyle modification) can potentially avert or delay the onset of DM (131; 132). See *'Section 1.2.3 Patients at Risk of Future DM and Prediabetic States'*.

Therefore, it is an important public health priority that patients with impaired glucose regulation (IGR) and at future risk of DM are properly identified and followed up regularly. This was exemplified by Vaccaro et. al. in a follow up study of 1300 study subjects over a 11 year period using 75g OGTT and FPG (133). Though the findings of the study supported the concept of IFG and IGT in predicting diabetes (9.1% in IFG, 32.5% in IGT and 44.4% in both IFG and IGT), an alarming 66.7% of patients who developed DM at the end had normal glucose tolerances at the start of the study. The results of this study highlight the need for regular screening of high risk patients groups despite normal glycaemic tolerances. In the UK, there are recommendations that patients with IGR should be screened at least annually (134) and high risk normoglycaemic patients every 2 years (18).

1.5.2 The effect of lifestyle and psychological stress to glycaemia:

Glycaemic control and the risk of future DM is closely linked to the daily well being and life choices an individual makes. Sedentary lifestyles and lack of exercise are known risk factors to develop DM in the future (135; 136). Glucose levels are influenced by diet, psychological stress, and physical activity (10; 137).

1.5.2.1 Lifestyle & Environment

Excessive weight or obesity increases the risk of impaired glucose regulation (IGR) and T2DM in all age groups (138). Obesity acts in part by inducing peripheral tissue insulin resistance, an important component of T2DM. The reason why more than two thirds of patients develop DM can be attributed to obesity. It is associated with a significant increase in the risk of cardiovascular mortality and is closely related with a sedentary lifestyle and a poor diet (138).

Dietary modification is an important behavioural aspect in the treatment of DM. Studies on nutritional therapy (with concurrent lifestyle intervention) have demonstrated almost a 2% fall in HbA1c in newly diagnosed patients with T2DM (139; 140). The metabolic benefits seen through dietary intervention is related both to caloric restriction and weight reduction (139). Caloric restriction regulates blood glucose excursions and hence reduces the risk of long term hyperglycaemic complications. On the other hand, weight reduction acts by improving liver function in non-alcoholic steatohepatitis; a condition linked with insulin resistance. The combined effect of these 2 interventions has been shown to decrease the risk of developing T2DM and improve glucose levels in patients with established disease (140).

Enhancing physical activity also benefits glycaemic control, an action which is independent of weight loss. All forms of physical activity, including aerobic, resistance or combined training are all equally effective in reducing glycaemic levels (141). Exercise leads to an increased responsiveness to insulin and can delay the

progression of IGR to T2DM (142). A meta analysis examining the effects of physical activity on patients with T2DM found that exercise training reduced HbA1c by 0.7% in the absence of significant weight loss (143).

The education of patients regarding diet and insulin therapy facilitates patient self management of DM. This enables patients to regulate their lifestyle, diet and adjust insulin therapy to suit their nutritional intake. The effect of providing a structured education programme to patients with DM has shown some favourable results in several studies (144; 145). A randomised controlled trial showed that T1DM patients attending the Dose Adjustment for Normal Eating (DAFNE) education program demonstrated glycaemic improvements (1% difference in HbA1c), higher patient satisfaction and an overall better quality of life after the program (146). Similarly, patients who attended the XPERT structured education program for T2DM patients exhibited similar findings 14 months later (144).

Patients with DM who receive intensive lifestyle intervention programmes (comprising the combined effect of dietary intervention, increased physical activity and behaviour modification) enjoy improvement to long term glycaemic control and quality of life (147; 148). The 1st year results in the Look AHEAD study of 5145 T2DM patients randomly assigned to receive intensive lifestyle intervention versus standard therapy reported a 8.6% versus 0.7% weight loss and a reduction of HbA1c from 7.3 to 6.6% versus 7.3 to 7.2% between the study groups respectively (149). Similarly, the Diabetes Prevention Program study showed that 58% reduction in the progression to T2DM in patients with IGR with intensive lifestyle intervention over 2.8 years.

Unfortunately, despite its obvious benefits, only a small proportion of patients with T2DM are able to maintain a lifestyle change successfully. In a 10 year study looking at regular exercise, compliance in patients fell from 80% at 6 weeks to <50% at 3 months and <20% at one year (142). Similarly, a prospective study by Close and

colleagues found that less than 40% of patients with DM ate within their prescribed diet (150).

1.5.2.2 Psychological stress

Psychological stress also plays an important role in influencing glycaemic control in patients with DM (151). The psychophysiological experience of stress triggers the release of counter-regulatory hormones which results in an elevation of blood glucose levels. Excess stress can also negatively influence self care, diet and physical activity causing further disruptions to glycaemic control (137).

Patients with DM are more prone to suffer from psychological stress due to issues directly related to their disease such as frequent capillary glucose testing, medication and lifestyle disruption (152). This puts them at risk of developing clinical depression which further interferes with their self care of DM.

It is therefore hardly surprising that stressors of long duration such as major life events (e.g. divorce) result in higher blood glucose levels , even after controlling for self care variables (153). Studies on victims of natural disaster have been shown to have greater stress levels, require greater health care input and have poorer care in the months following the event (154-157).

Psychological intervention in patients with DM has been shown to improve glycaemic control and reduce psychological distress in some but not all studies (158). A systematic review of 12 studies looking at patients with T2DM randomly assigned to psychological intervention or conventional treatment noted that mean HbA1c was 0.76% (CI -1.32 to -0.18) lower in the intervention group (158). This group also had less psychological distress as compared to those conventionally treated despite no difference in weight control between the 2 groups.

Chapter 1:

Part 6: Other measurements of glycaemic control:

1.6.1 Introduction:

An accurate and reliable assessment of glycaemic control is essential and can aid clinicians in tailoring glycaemic therapy to reduce the risks of hyperglycaemia and avoid treatment complications such as hypoglycaemia. Diabetes monitoring at the present time is currently managed by a combination of daily self monitoring of blood glucose (SMBG) and regular assessments of HbA1c (159).

This part of the chapter discusses some of these methods in assessing glycaemic control. Initial discussion surrounds the more commonly used method of self monitoring of blood glucose (SMBG) using capillary glucose strips. This is followed by discussion surrounding the less commonly used measurement methods using continuous glucose monitoring devices (CGMS), glycated albumin and fructosamine.

1.6.2 Self monitoring of blood glucose (SMBG):

Monitoring of glucose in patients with DM before the early 1980s was limited mostly to measuring glucose concentrations in urine samples. Unfortunately, this measurement method was unreliable, primarily due to the differing test strip sensitivities and variations of intersubject renal glucose thresholds (160).

Although the measurement of blood glucose was possible in a laboratory setting many years before the 1970s, portable measurement of blood glucose was only available after this period following the introduction of ‘dry’ chemistry techniques which used the blood sample itself as a solvent for the measurement of glucose.

Early studies looking at self monitoring of blood glucose (SMBG) versus conventional urine measurements showed better glycaemic control and a significant reduction of hypoglycaemia rates in insulin treated patients (161). These encouraging results spurred further advancement in this area ultimately leading to the production

of cheaper, more portable and increasingly more accurate devices over the years (160; 162).

At this present time, SMBG has become an integral part in the treatment regimen of patients on insulin therapy. The American Diabetes Association (ADA) recommends that patients with T1DM on multiple injections carry out SMBG at least 3 times a day and T2DM patients on insulin or hypoglycaemic agents at least daily (30). Monitoring levels via SMBG in this patient group enables them to make adjustments for insulin and diet content based on the 'real time' glucose values which can improve overall glycaemic control and potentially avert hypoglycaemic attacks. Furthermore, numerous clinical studies that have shown the benefits of tight glycaemic control consistently include regular SMBG monitoring as part of the multifactorial interventions required for this treatment regime (30).

In contrast, studies on T2DM patients not on insulin therapy have failed to find a positive link between SMBG and improved glycaemic control (163-165). The 2007 Freemantle study of 1286 T2DM patients over 5 years failed to show that either SMBG testing or its frequency was associated with an improvement in glycaemic control regardless of treatment (163). A study by Farmer et al in the same year reported that non insulin using patients with T2DM had no improvement in glycaemic control using SMBG despite being given training and encouragement (164). In line with this, a UK cost analysis with a consideration to quality of life found that regular SMBG in patients with DM not on insulin was unlikely to be cost effective (165).

However, not all studies have shown this lack of benefit. A meta-analysis performed 5 showed that regular SMBG resulted in a mean reduction in HbA1c of 0.4% in 1307 non insulin using patients with T2DM (166). Furthermore, an argument to most of the study results on the efficacy of SMBG and glycaemic control lies in its methodology. Many of the RCTs performed and meta-analysis include other interventions (including dietary advice and drug initiation) thereby making it difficult to assess the independent contribution of SMBG on glycaemic control. In line with this, patients

who comply with self monitoring may potentially have better lifestyle compliance or have worse glycaemic control and hence are more motivated. Patients who are less motivated may not be willing to take part in research and therefore study results may be only representative of a particular group of patients (167).

In spite of this, at the present time, there is a lack of conclusive evidence to show any benefit in using SMBG on T2DM patients not on hypoglycaemic or insulin therapy. Current guidelines do not support routine use of SMBG in this group of patients but may still serve ‘as a guide to the success of therapy’ in selected patients (30).

SMBG is the assessment of ‘real time’ glucose levels and hence its application in clinical practice is inherently limited by its inability to accurately represent long term glycaemic control unless multiple regular readings are taken over an extended period of time. The utility of SMBG in clinical practice is mostly as an adjunct to HbA1c measurements and is not recommended as the sole method of measuring glycaemic control.

1.6.3 Continuous Glucose Monitoring System (CGMS):

Continuous Glucose Monitoring Systems (CGMS) have the ability to sample glucose levels continuously and can provide detailed information on the glycaemic status of an individual when used concurrently with SMBG. Data obtained via CGMS monitoring provides a plot of an individual's glucose whilst allowing input of events such as physical activity, meals and finger prick SMBG checks (171).

CGMS measures the glucose content of interstitial fluid using an electrochemical enzymatic sensor. This is achieved by inserting a needle sensor subcutaneously or by implanting the entire device subcutaneously. Newer devices can provide patients with nearly real time results of their glucose on a continuous basis and can reflect relatively quick changes of blood glucose (within 5-12 minutes) (172).

However, despite its technological capabilities, compared with regular SMBG monitoring, the superiority of CGMS is not well established in clinical practice. Firstly, CGMS use is limited as it is much more expensive. Furthermore, CGMS does not preclude SMBG monitoring as sensor results regularly require calibration with SMBG, and the latter is still recommended for making acute treatment decisions due to the delay in blood glucose changes being detected by CGMS (10). However, newer CGMS devices have alarms for hypo and hyperglycaemia and can be useful in alerting patients to changes in blood glucose before they have reached critical limits.

The clinical efficacy of CGMS in patients with DM has come up with variable conclusions. Regular use of CGMS has been shown to improve physical activity and decrease weight (173). Studies have shown improvements to hypoglycaemia prevention and statistically insignificant improvements to glycaemic control (202-204). A study on the continuous use of CGMS over 3 months did show significant improvements to glycaemic control compared to SMBG and intermittent CGMS (twice for 3 day periods every 2 weeks) (174). However, continuous CGMS in this study group meant that the device in this case was nearly continuously kept on throughout the study period of 3 months making it a very expensive and impractical method to be applied in clinical practice.

Reviews on the use of CGMS have concluded that at the present time, there is insufficient evidence to support the use of this device above SMBG in reducing HbA1c but may have a role in the detection and reduction of hypoglycaemic episodes and hypoglycaemic unawareness (10; 175). CGMS remains an evolving technology and at the present time provides the best method of enabling clinicians and patients to discern glycaemic control relatively accurately and continuously over a period of time. It is by no means ideal due to its numerous limitations but increasingly, emerging data suggests that it may offer benefit to patients who are willing to wear it most of the time.

1.6.4 Fructosamine & Glycated albumin:

The term fructosamine is used to describe the sum of all ketoamine linkages as a result of glycation of circulating serum proteins (176). The degree of glycation of proteins are used as a basis of the assessing the degree of glycaemia. The turnover of protein is more rapid than haemoglobin and thus fructosamine concentrations represents mean blood glucose levels over a much shorter time frame. (1-2 weeks) compared to the HbA1c (30).

Recently, there is an increasing amount of attention being focused on the use of glycated albumin (GA) as an indicator of glycaemic status. Albumin is the largest component of all the plasma proteins representing 80% of the total molecules and 60% of the total plasma protein concentration. The half life of GA is approximately 12-19 days (177).

Studies have shown that fructosamine and glycated albumin correlate well with HbA1c and have been suggested as alternative methods of measuring glycaemic control, particularly when HbA1c values are unreliable (39; 40).

However, at the present time, fructosamine and glycated albumin measurements in clinical practice remain limited. One of the main reasons for this is that though the levels of these measurements correlate well with glucose levels, the relationship to a therapeutic glycaemic target goal has not been established (178). Most clinical trials and studies have measured HbA1c values as the goal standard measurement for glycaemic control and therapeutic targets. There is scant data on the use of other measurements as an indicator of glycaemic control. Therefore, whether GA or fructosamine is an accurate predictor of morbidity and mortality in patients with DM remains to be ascertained (30).

In parallel to this, as GA and fructosamine are reliant on plasma proteins, its values may need to be readjusted under conditions where serum albumin levels are abnormal (179). For instance, patients with cirrhosis of the liver and the nephrotic syndrome have lower values of fructosamine (179; 180). Even normoalbuminaemic with T1DM have shown diurnal variation in fructosamine levels which is closely related to protein levels (180). Similarly, in patients on peritoneal dialysis or with massive proteinuria in CKD, glycated albumin values are lower due to the shorter exposure time of albumin to glucose in the plasma (181).

Furthermore, GA values have recently been shown to be influenced by other biochemical indices including hyperuricaemia (182), raised alanine transaminase in non-alcoholic liver disease (183) and hypertriglyceridaemia (184). Another unusual but consistent finding has been observed whereby obese diabetic patients and non-diabetic individuals have been found to have lower fructosamine and GA values than lean ones (185-188).

An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

Chapter 2:

The effect of severe disruptions to lifestyle and stress following a flooding disaster on the glycaemic control of patients with DM in a UK population.

2.1 Introduction:

In the last 30 years, water related disasters have increased four fold, affecting more than 2.6 billion people and causing more than 200,000 deaths (189; 190). Previous data suggests that there is a significant deterioration in standards of health during times of disaster in both availability and accessibility to healthcare (154). This can affect the quality of life of the victims particularly the mental and psychosocial aspects which can continue long after the event.

The long term effects of flooding on psychological health can be more important than illness or injury (154). In patients with diabetes, natural disasters have been shown to have a negative impact on glycaemic control and provision of health care (191-193). With particular regard to flooding, diabetes patients affected by Hurricane Katrina in New Orleans showed only a modest overall 0.1% rise in HbA1c in samples taken 6-16 months after the event compared to those taken 6 months before (191). However, by not being able to examine the period in the immediate aftermath of the disaster, it is possible that an acute change in HbA1c due to stress and anxiety could have been missed (152; 194).

In June 2007, the city of Hull and of surrounding East Yorkshire in the United Kingdom experienced torrential flooding and, though not alone at that time, was the region with the largest number of households and people affected in any one area of the UK (195). Over 8,600 households were damaged and over 20,000 people (from a total population of approximately 500,000) affected. Six thousand three hundred of these people were forced to live in alternative accommodation with more than 1,400 living in caravans. Houses were uninhabitable for many months and after 1 year there were still 1,476 households living in temporary accommodation. Lifestyle, eating habits and the living environment were significantly altered in those affected (196).

This study was undertaken to establish whether the glycaemic control of the population of patients with diabetes mellitus (DM) was affected by the flooding at that time. It was hypothesised that the disruption to lifestyle and increased stress would result in the worsening of glycaemic control of individuals with DM affected

by the floods. The effect of the floods was determined by comparing the HbA1c of those flooded with those who were unaffected in the year prior and the year following the event. Particular regard was paid to the different treatment groups of the patients involved.

2.2 Methods

Participant identification:

Questionnaires were sent out in December 2008 to the 15,846 patients over the age of 18 years registered as having Type 1 or Type 2 DM according to the diabetes records on the laboratory computer serving the entire region.

The questionnaires were sent out in a double sided single page containing 20 items. Participants were invited to complete details including whether and how they were affected by the floods, their duration and type of diabetes and their drug treatment in July 2007. All questionnaires were assigned a unique code number specific for each patient which enabled identification and correlation of results. Analysis was performed on those questionnaires returned by the 31st of March 2009.

Exclusion criterion:

We excluded all participants who died over this period and any participant who was newly diagnosed with DM after December 2006.

Questionnaire production & distribution:

The questionnaires were designed and analysed using FORMIC© data capture software (FORMIC software, Heathrow, United Kingdom). All questionnaires and study envelopes were printed and sent out via Kallkwik (Hull, UK) printer services.

The study envelope contained a patient information sheet, a stamped return envelope and two A4 sheets separated by a serrated edge to enable easy parting. The first of the A4 sheets contained a single sided cover letter with an introduction and explanation to the nature of the study. The next was double sided questionnaire inquiring into the

respondent's involvement in the flooding disaster (if any) and specific details of their diabetes mellitus including treatment and duration of disease.

To protect confidentiality in the event the questionnaire was lost or misplaced, the following steps were taken.

1) Participants of the study were requested to separate the cover letter (which contained their name and address) from the questionnaire via the serration and return it to the research team using the stamped addressed envelope.
2) All study subjects who were asked to participate were only identifiable to the study team via 5 digit patient specific number present in the right top corner.

A copy of the questionnaire is available in pg 71-73. (Figure 1)

Questionnaire and statistical analysis:

The participants in the study reported their involvement, or not, in the floods. They were considered affected by the floods if they had property damage which may or may not have necessitated them to move from their homes and/or if they had to accommodate friends or family who were affected over this period. Participants were considered not to have been affected if they did not report any involvement in the floods over the period of the study. Further questions included details of diabetes diagnosis and current treatment. Diabetes patients were consulted on the format of the questions before it was distributed. All questionnaires which were not spoiled were included in the analysis.

All questionnaires which were not spoiled were included in the analysis. The participant's unique questionnaire number was then used to match their responses to their laboratory data.

Laboratory Results:

Laboratory results from participants were obtained for the year before and the year after the flooding (from 1st Jul 2006 until 30th June 2008). All HbA1c measurements were analysed using the same DCCT aligned ion-exchange chromatography analysers (Menarini HA-8160 HbA1c, A. Menarini, Berkshire, United Kingdom) in the same laboratory located in Hull Royal Infirmary.

All the patient identification numbers from the respondents were correlated to their corresponding patient hospital numbers. Using this information, patient details such as age, gender and biochemical results such as glycated haemoglobin (HbA1c) were extracted from the LabCentre database (Clinisys, Surrey, UK) into Microsoft Excel.

Statistical methods:

Baseline demographics were compared by the t-test (continuous data) or Chi-squared test (categorical data). The number of HbA1c measurements was analysed using Poisson regression. A common problem encountered with Poisson regression is overdispersion, where the variance of the outcome measure is greater than its mean (197; 198). There was no evidence of overdispersion. HbA1c was measured repeatedly over the duration of the study (2-years) giving rise to longitudinal data. The analysis of longitudinal data has developed greatly over the last 20 years (199) with many options available. Here, we used the generalized estimating equation (GEE) with identity link and normal errors to assess the effect of HbA1c levels over repeated time-points in participants both affected and unaffected by the floods. Such models allow for the correlation between successive measurements on the same subject to be taken into account. P-values were calculated from Wald robust standard errors (i.e., using the empirical (information sandwich) variance estimates). The reference group for comparisons was taken as April-June 2007 (i.e., three months before the floods). An arbitrary level of 5% statistical significance (two-tailed) was assumed. The Stata statistical computer package (version 10) was used to analyse the data (StataCorp, 2007).

Power calculation:

Twelve months of HbA1c data prior to the 2007 floods was used to estimate any HbA1c change which could be detected by this study. A two group t-test with a 0.01 two-sided significance level would have 80% power to detect the difference between a mean HbA1c amongst unaffected patients of 7.8% and a mean of 8.1% amongst those who were flooded, a difference in means of 0.30%, assuming a common standard deviation. This effect applied when the sample sizes in the two groups were 3000 amongst unaffected and 300 amongst those flooded (a total sample size of 3300).

From the known prevalence of diabetes in the population (6%) it was estimated that 900 with diabetes were likely to have been flooded. This required a response rate of 23% from patients not flooded, and a 33% response amongst those who were, in order to meet the power calculation criteria.

Ethics:

Ethical approval was obtained from the Hull and East Riding and South Humber Local Research Ethic Committee (Ref no 08/H1304/83) prior to commencement of the study. This study was also registered under the UK Clinical Research Network (UKCRN) (DRN299). For questionnaire production and analysis of the data, the FORMIC fusion for healthcare software™ was used.

2.3 Results

Responders

One thousand eight hundred and fifty six were returned (11.7% of those sent). Of this number, 50 questionnaires were not included in the analysis due to the questionnaire being unintentionally or intentionally spoiled. A further 63 participants who were diagnosed with DM over the period of the floods in the region were also excluded.

This left 1743 patients (296 flood affected) who had 6213 HbA1c measurements (1072 affected) over the study period. Median affected 4 visits (25%, 75% centiles 3,5),

maximum 8; median unaffected 3 visits (3,4), maximum 8. There was no significant difference in frequency of HbA1c measurements between the two groups using Poisson regression analysis.

Of the 296 patients flooded, 273 (92.2%) had direct damage to their belongings and property of which 85 (28.7%) were forced to move out of their household. The remaining 23 (7.8%) patients did not have direct damage to their property but had to accommodate family and friends who had to move due to the floods.

The models quoted are unadjusted. Inclusion of age and sex made little difference to our conclusions. Other characteristics of these 2 groups are shown in Table 6.

Non-response bias

A total of 13990 questionnaires were not returned. There were 7231 men (52%) and 6759 women (48%). Non-responders were more likely to be male, younger and have higher HbA1c values (mean HbA1c 12 months prior) than responders, albeit the absolute differences were not marked (non responders vs. responders; 52% vs. 43% male, $p<0.001$ using chi squared; age (mean ± SD) 65.5 ± 16.4 vs. 65.9 ± 13.5 years, $p =0.02$, HbA1c 7.8% (95% confidence interval) (7.7,7.9) vs. 7.6% (7.5,7.7), $p<0.001$ respectively (t test))

HbA1c 12 months before and after flooding:

Mean HbA1c in the group unaffected by flooding was no different in the 12 months before compared to the 12 months after the event (7.5% (7.4,7.6) vs. 7.5% (7.4,7.6), t test $p=0.46$). In contrast, there was a significant deterioration in glycaemic control in the group of participants affected by flooding (mean HbA1c 7.6% (7.5, 7.7) vs. 7.9% (7.7, 8.0), $p=0.002$). This rise was mainly a consequence of the change amongst flood victims who were taking insulin treatment 8.2% (8.1, 8.3) vs. 8.6% (8.3, 8.9), $p=0.002$) rather than treated by lifestyle and oral agents (7.1% (7.0, 7.2) vs. 7.2% (7.1, 7.2), $p=0.17$).

In the insulin treated group, similar findings were noted in the patients with type 1 (T1DM) and type 2 diabetes (T2DM) affected by the floods. Patients with T1DM (n = 60) had an increase in HbA1c (8.1% (7.9, 8.5) vs. 8.6% (8.2, 8.9), p=0.02) as did the patients with insulin treated T2DM (n = 50, HbA1c 8.2% (7.9, 8.4) vs. 8.6% (8.3, 8.8), p=0.04). Type 1 patients (n=234) and insulin treated type 2 patients (n=248) unaffected by the floods showed no such change (T1DM 8.2% (8.0, 8.4) vs. 8.1% (7.9, 8.2), p=0.08: T2DM 8.2% (8.1, 8.3) vs. 8.1% (8.0, 8.2), p=0.27).

HbA1c values using GEE analysis:

The figure 1A-C show mean Hba1c values (95% confidence intervals) for the quarterly time-points estimated from the GEE regression models. Means are unadjusted for covariates. P-values are shown as asterisks (** $p<0.001$; * $p<0.05$) taking April-June 2007 as the reference group for comparisons. The largest and most statistically significant increase from the reference HbA1c values was 6-9 months after the floods and exclusively in the 3 groups of patients affected by the event. (Figure 2A-C and Table 7). In the 3 months before the April-June 2007 reference period, the HbA1c among patients subsequently unaffected by the flooding was statistically (but inexplicably) significantly higher (0.1% in all patients and 0.3% in insulin treated ones).

2.4 Discussion:

This study has shown a significant deterioration in glycaemic control amongst diabetes patients in the year following the flooding in Hull and East Yorkshire. It was most pronounced in patients treated with insulin and peaked in the period 6-9 months following the incident before returning back to previous levels by 12 months. This study therefore supports the few previous studies that also showed a worsening of the health of patients with diabetes mellitus post natural disaster (191; 192).

This data also adds to the recently published study describing the effect of Hurricane Katrina on several aspects of diabetes care (191). Due to the difference in the nature

and scale of the Katrina disaster, the laboratory data in that study dated from 6 months following the disaster. In contrast, since the main hospitals and most General Practitioner health centres were unaffected by the Hull floods, it allowed this collection to take place immediately after the event and so give an insight into the time course of HbA1c in these first few months. The questionnaire also allowed a comparison between patients who were affected with those in the same area who were unaffected. Additionally, the effect of flooding on particular treatment groups could be examined.

Since changes in HbA1c lag behind those of blood glucose by 8-12 weeks (31), the current results suggest that the disruption in glycaemic control occurred almost immediately following the floods and was then sustained for about 6 months following the event.

The time course of the rise and fall in HbA1c in this study after flooding is in contrast to the experience post-Katrina (191). In that disaster HbA1c seemed to continue to rise for at least 2 years after the event, whereas in the UK it was back to baseline levels within a year. This observation could either be due to the comparatively prolonged and more severe impact of Katrina in the diabetes population or may be a reflection on the importance of having functional healthcare to support patients after such a disaster.

In the Katrina disaster the change in HbA1c was most prominent in the disadvantaged and uninsured patients with DM whilst the difference was modest in all other groups of patients (191). Socioeconomic factors were not evaluated in the present study, so confirmation of this finding could not be made. However, what was notable was that the rise in HbA1c was especially marked in the insulin treated group affected by the floods. This observation is surprising, given the fact that insulin treated individuals can potentially alter insulin dosages to suit any changes in glycaemic control due to their circumstances as compared to individuals treated with oral agents and/or lifestyle who are less likely to titrate their medications to suit these changes. Possible

reasons that could explain these findings includes the reduced frequency in blood sugar monitoring, a lack of education in managing glucose and insulin therapy and/or the change in lifestyle over the periods after the flooding disaster.

The findings of this study are important as even relatively short periods of poor glycaemic control have been found to have long-lasting detrimental effects on the development of diabetes complications (95; 96). Moreover, having more variable 3 monthly HbA1c has also been found to compound microvascular complication risk (200), so the rise and fall in HbA1c found in flooded patients may have been more detrimental than even their average rise suggests.

Also surprising was the fact that although glucose control seemed to suffer as a consequence of the flooding, attendance for diabetes review (as evidence by the number of HbA1c measurements) did not. It may, of course, be that it was the rise in these measurements, and their identification, that helped prompt their subsequent fall.

A strength of this study was the fact that the whole area involved was served by a single laboratory. The large number of patients involved in this analysis should also have been a strength, but this is tempered by the poor response rate which means a non responder bias could potentially limit the generalisability of the findings (201). Nonetheless, given the estimated number of patients expected to have been affected by flooding, the proportion of flooded patients responding is, perhaps predictably, likely to have been much higher than from those unaffected..

From a practical point of view, this study has highlighted ways in which diabetes care could be improved if such an event was to recur. Firstly, it confirmed that in the year following flooding there is significant deterioration in the glycaemic control of diabetes patients compared to the preceding year. While it was reassuring that patients not taking insulin therapy experienced little in the way of HbA1c rise, those on insulin treatment may require particular targeting from diabetes healthcare professionals following a disaster. The time period of support, in this case for between 6 and 9 months after an acute flooding event, is crucial for planning for health care provision and disaster planning in event of a recurrence of this type of catastrophe. Indeed, this

study may also have shown the need for more patient education on insulin dose titration and dealing with fluctuations in glycaemic control which, if successful, may have helped minimise the HbA1c rise that was found here.

It is essential that everyone involved in the provision of medical care is made aware of the health needs of the victims who have been affected by flooding, the consequences of which can continue for months following the event (154). This study has shown the need for patients with diabetes, particularly those on insulin treatment, to be regarded as a priority group in any future disasters.

Table 4: Changes in glycaemic control in respondents affected by flooding

Affected	Mean HbA1c (%) before floods (12 months)	Mean HbA1c (%) after floods (12 months)	P*
All	7.6 (7.5, 7.7)	7.9 (7.7, 8.0)	0.002
Affected patients on insulin therapy	8.2 (8.1, 8.3)	8.6 (8.3, 8.9)	0.002
Affected patients on lifestyle/OHAs	7.1 (7.0, 7.2)	7.2 (7.1, 7.2)	0.17

* 2 tailed paired t test

Table 5: Changes in glycaemic control in respondents unaffected by flooding

Unaffected	Mean HbA1c (%) before floods (12 months)	Mean HbA1c (%) after floods (12 months)	P**
All	7.5 (7.4,7.6)	7.5 (7.4,7.6)	0.46
Unaffected patients on insulin therapy	8.2 (8.0, 8.4)	8.1 (7.9, 8.4)	0.05
Unaffected patients on lifestyle/OHAs	7.1 (6.9, 7.2)	7.2 (7.0,7.3)	0.34

* 2 tailed paired t test

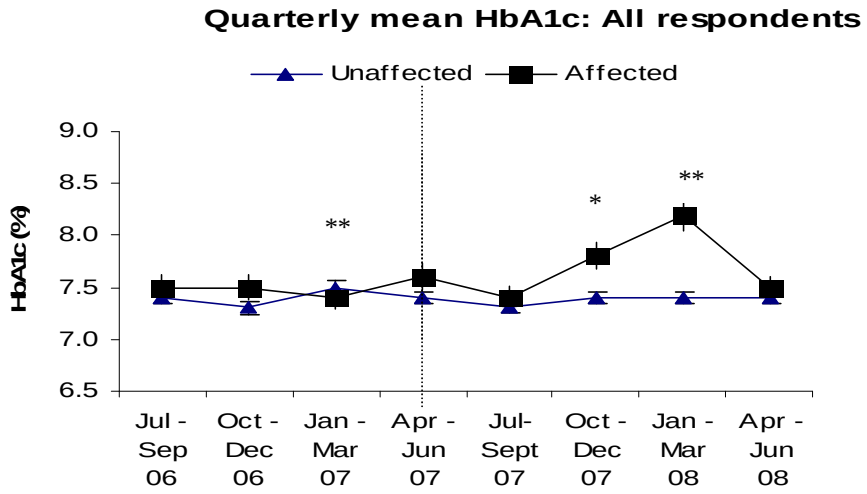
Table 6: Baseline characteristics of study group.

	Affected	Unaffected	p value
Gender	164 women 132 men	820 women 627 men	0.71 [a]
Type 1 Diabetes	60	234	0.11 [a]
Type 2 Diabetes	236	1213	
Age (mean ± SD years)	65.4 ± 13.5	66.3 ± 13.4	0.27 [b]
Duration of diabetes (median) (25^{th},75^{th} centiles)	10 (5,20)	10 (5,21)	0.66 [b]
Number of HbA1c per participant before flood (median) (25^{th},75^{th} centiles)	2 (1,2) n = 531 samples	2 (1,2) n = 2569 samples	0.20 [c]
Number of HbA1cs per participant after flood (median) (25^{th},75^{th} centiles)	2 (1,2) n= 541 samples	2 (1,2) n=2572 samples	0.25 [c]
On insulin treatment	110 (37%)	482 (33%)	0.36 [a]
Diet and Lifestyle	70 (23%)	394 (27%)	0.24 [a]
Metformin	163 (55%)	751 (51%)	0.27 [a]
Sulphonylurea	100 (34%)	449 (30%)	0.27 [a]
Thiazolidenediones	23 (7%)	256 (18%)	<0.001 [a]
GLP-1 analogues	7 (2%)	26 (2%)	0.48 [a]

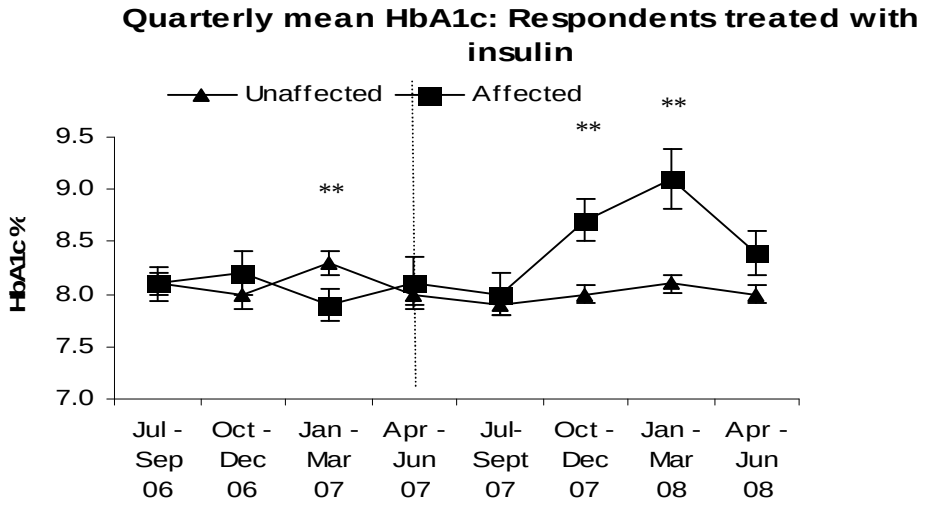
[a] chi squared test
[b] unpaired t test
[c] Poisson regression. (Poisson assumption (mean=variance) met.)

Figure 2: Comparison of mean quarterly HbA1c values in flood responders affected and unaffected by flooding

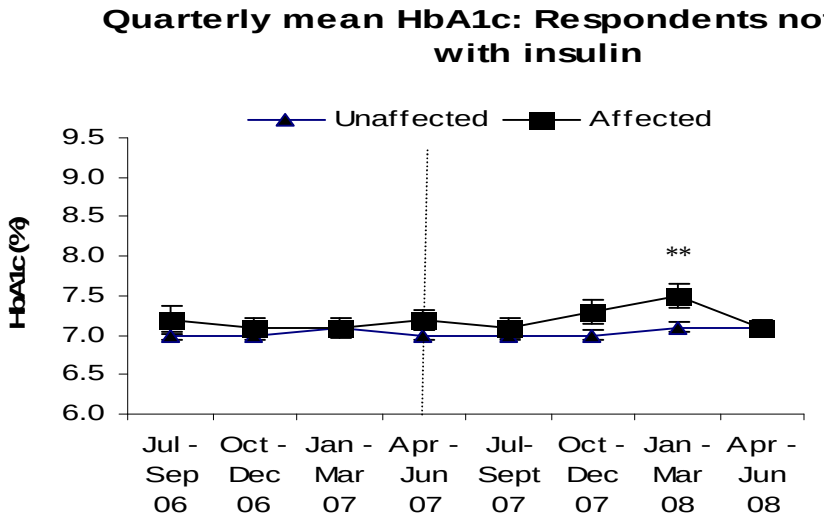
2A



2B



2C



Figures 2A-C Quarterly HbA1c values in patients on different therapies involved in the effect of flooding and patients with DM. Distribution of mean HbA1c and standard error quarterly over 12 months before and after floods. Mean HbA1c in all participants (1A), in insulin treated participants only(1B), and in those treated by lifestyle/oral agents (1C)
Dark squares(■) : participants affected by flooding. Dark triangles(▲): participants unaffected by flooding.
The dotted line (……) marks the reference period in which comparison of HbA1c values were made. * $p < 0.05$ ** $p<0.001$

Table 7: Quarterly mean HbA1c values over 12 months pre and post floods

Date	Mean HbA1c (%) unaffected by flooding	p-value	Mean HbA1c (%) affected by flooding	p-value
All patients				
Jul–Sep 2006	7.4 (7.3,7.5)	0.7	7.7 (7.5,7.9)	0.68
Oct–Dec 2006	7.4 (7.3,7.5)	0.54	7.5 (7.3,7.7)	0.90
Jan–Mar 2007	7.5 (7.4,7.6)	<0.001	7.4 (7.2,7.6)	0.05
Apr-Jun 2007	7.4 (7.3,7.5)	Reference	7.6 (7.4.7.8)	Reference
Jul–Sept 2007	7.3 (7.2,7.4)	0.25	7.4 (7.2,7.6)	0.23
Oct–Dec 2007	7.4 (7.3,7.4)	0.83	7.8 (7.6,8.0)	0.02
Jan-Mar 2008	7.4 (7.3,7.5)	0.15	8.2 (8.0, 8.4)	<0.001
Apr-Jun 2008	7.4 (7.3,7.5)	0.27	7.5 (7.4,7.7)	0.91
Insulin Treated				
Jul–Sep 2006	8.1 (7.9,8.2)	0.77	8.1 (7.7,8.4)	0.71
Oct–Dec 2006	8.0 (7.9,8.2)	0.95	8.2 (7.8,8.6)	0.86
Jan–Mar 2007	8.3 (8.2,8.5)	0.001	7.9 (7.6,8.3)	0.24
Apr-Jun 2007	8.0 (7.9,8.2)	Reference	8.1 (7.8,8.5)	Reference
Jul–Sept 2007	7.9 (7.7,8.1)	0.18	8.0 (7.6,8.4)	0.473
Oct–Dec 2007	8.0 (7.9,8.2)	0.95	8.7 (8.4,9.1)	0.003
Jan-Mar 2008	8.1 (7.9,8.2)	0.67	9.1 (8.8,9.5)	<0.001
Apr-Jun 2008	8.0 (7.9,8.2)	0.92	8.4 (8.0,8.7)	0.18
Not insulin treated				
Jul–Sep 2006	7.0 (6.9,7.1)	0.76	7.2 (6.9,7.4)	0.91
Oct–Dec 2006	7.0 (6.9,7.1)	0.53	7.1 (6.9,7.3)	0.64
Jan–Mar 2007	7.1 (7.0,7.2)	0.11	7.1 (6.8,7.3)	0.50
Apr-Jun 2007	7.0 (6.9,7.1)	Reference	7.2 (6.9,7.4)	Reference
Jul–Sept 2007	7.0 (6.9,7.1)	0.75	7.1 (6.8,7.3)	0.62
Oct–Dec 2007	7.0 (6.9,7.1)	0.86	7.3 (7.0,7.5)	0.42
Jan-Mar 2008	7.1 (7.0,7.2)	0.12	7.5 (7.3,7.7)	0.008
Apr-Jun 2008	7.1 (7.0,7.2)	0.09	7.1 (6.8,7.3)	0.43

Hull and East Yorkshire Hospitals NHS
NHS Trust

Diabetes Centre
PO Box 490
Hull & East Yorkshire Hospitals NHS Trust
HU9 9GE

Dear Sir/ Madam

The flood that hit Hull last June had a devastating effect on people's lives and on their health. We would like to invite you to take part in a research study to see how the floods have affected people with diabetes. This is so that we can plan for such a disaster in the future should the same circumstances occur and also give guidance and help to other parts of the country should they be affected. More detailed information can be obtained from the information leaflet enclosed.

If you do not wish to take part, thank you for reading this letter and please do not fill or return any part of this.

If you agree to take part, we would be very grateful if you could fill out the enclosed questionnaire and return it in the self addressed envelope in this letter. We may need to look up your previous laboratory results to help us with our study. The individual information you provide and your lab results will not be disclosed in any final report.

Even if you were not affected by the flooding, it is still important to us if you could also take the time to fill out the form if you have diabetes. If you do not have diabetes, please accept our apologies and please do not fill or return this questionnaire.
There is a self addressed envelope and filling it should take you no more than 5 to 10 minutes of your time. Thanking you in advance.

If you have any other queries, please do not hesitate to contact us on: Clinical Research Centre Brocklehurst Centre
Tel: 01482 675302/675372

Were you affected by the floods in Hull? (tick all that apply) Patient ID 0 6 4 2 9

- ☐ Yes. We suffered damage to our belongings and property
- ☐ Yes. We had to accomodate our friends and relatives in our house during this time
- ☐ Yes. I only found out much later on that I had damage to my property or had to accomodate my friends/family later on
- ☐ No. I was not affected

Did you have to move from your home because of the floods?

☐ Yes ☐ No

If yes, how long did you have to move out for?

☐ Less than 3 months ☐ Between 6 and 12 months

☐ Between 3 and 6 months ☐ More than 1 year

How much did the floods affect your physical health? (1 very little, 10 very much)

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

How much did the floods affect your mental health? (1 very little, 10 very much)

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

How much did the floods affect your diabetes? (1 very little, 10 very much)

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

How many years have you had diabetes? ☐☐

How was your diabetes first treated?

☐ Insulin injections ☐ Diet/lifestyle or tablets

If on insulin, how many years have you been on it? ☐☐

If you are not on insulin, what treatment are you on for your diabetes now? (tick all that apply)

- ☐ Metformin/Glucophage
- ☐ Avandamet
- ☐ Glicazide/Glipizide/Glimeperide
- ☐ Exenatide
- ☐ Pioglitazone/Actos/Rosiglitazone/Avandia

Did you feel that there was a change in your blood sugars during this period?

☐ Yes ☐ No ☐ Don't know

If you monitor your own blood sugars, did you have trouble monitoring it during the times of the floods?

☐ Yes ☐ No ☐ Don't monitor

If yes, were your blood sugars

☐ Higher ☐ Lower ☐ More unstable

Compared to the time before the floods, were you able to take your medications as regularly as usual?

☐ Less regularly ☐ As regular as normal ☐ More regularly

If you were not able to take your medications as regularly, what was the reason? (tick as many boxes as applies)

☐ Insulin/medication destroyed ☐ Unable to access prescription

☐ Unable to access GP ☐ Other cause: please state

Did your weight change following the flooding?

☐ Increased ☐ Decreased ☐ No change ☐ Don't know

Did the floods affect you in the way you had your meals?

☐ Affected the way I shop ☐ Affected my eating patterns

☐ Affected the way I store my food ☐ Affected the way I cooked or stored my food

☐ Other: Please state:

Did you have trouble getting medical advice during this period?

☐ Yes ☐ No

How often compared to before the flood did you need to see your GP?

☐ More often ☐ The same ☐ Less often

Did you have difficulty attending your regular diabetic appointments the year following the flooding?

☐ Yes

☐ No

If you had an appointment and were unable to attend, why do think this was? (tick all that apply)

☐ Moved address (letter sent to wrong address) ☐ Letter destroyed lost during flood

☐ Forgot ☐ Transportation problems

☐ Other: please state

If yes, please state why (tick all that apply)

☐ Forgot due to stress of floods ☐ Meter damaged of lost

☐ Other: please state

Do you think your diabetes control is back to normal?

☐ Yes ☐ No

If yes, how long do you think it took for it to return to normal? (months) ☐☐

Please tick here if you do not wish to be contacted by us again. ☐

An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

Chapter 3:

The effect of iron and erythropoietin stimulating agent therapy (ESA) on glycaemic control and its measurements.

Part 1:

Effect of intravenous iron and erythropoietin stimulating agent therapy (ESA) on glycaemic control and HbA1c measurements.

3.1.1 Introduction:

Glycated haemoglobin (HbA1c) is the most widely accepted and used method of assessing chronic glycaemia in patients with diabetes mellitus (DM). It is formed by the irreversible binding of glucose to haemoglobin over the life span of the red blood cell (30; 32).

Patients with chronic kidney disease (CKD) are commonly anaemic due to a variety of reasons, including functional or absolute iron deficiency and erythropoietin insufficiency (118; 238). Treatment of anaemia in patients with CKD using iron replacement therapy and erythropoietin stimulating agents (ESA) has resulted in significant improvements to quality of life and the correction of anaemia without the need for blood transfusions (110; 113; 202).

There are several studies that show a fall of HbA1c in patients treated with ESA and iron therapy (203-205). These studies are mostly in patients already receiving haemodialysis and those without DM. The effect of the lowering of the HbA1c values following either treatment has been postulated to be secondary to formation of new erythrocytes in the blood stream causing a change of proportion in young to old cells and also from an alteration in the red cell glycation rates (206; 207).

Despite this, a comprehensive analysis of the relationship between glycaemic control and HbA1c changes in patients undergoing both iron and ESA therapy has never been performed using robust methods such as 7 point daily capillary glucose monitoring (7PGM) or using continuous glucose monitoring (CGMS) devices. Thus, any class effect that iron therapy and ESA may have on HbA1c values could in fact represent a parallel change to glycaemic control along with the currently postulated physiological

changes. Furthermore, the effect of the fall in HbA1c following these 2 therapies have not been well studied in patients not already on haemodialysis.

This study therefore sought to establish how intravenous iron and ESA therapy influence HbA1c values in patients with Type 2 diabetes mellitus (T2DM) and chronic kidney disease not on haemodialysis. Robust monitoring of blood glucose was performed throughout the study period to determine if the anticipated fall in HbA1c was a true reflection of glycaemic control.

3.1.2 Methods:

This was a prospective study of patients with T2DM and CKD stage IIIB or IV (estimated glomerular filtration rate MDRD 15-44 ml/min/1.73 m^2) selected for treatment with intravenous iron and/or erythropoietin stimulating agents between January 2009 and December 2009 inclusive. All patients were attending a single renal service where the decision to commence iron and ESA therapy was made by the attending physician.

The study consisted of 2 groups. The first group (A) were patients selected for iron therapy according to clinical need and the second group (B) of patients were those needing ESA treatment. Glycaemic control in both patient groups was assessed in the month leading up to treatment and once again for a 4 week period 4 months after therapy. These assessments comprised the measurement of glycated haemoglobin (HbA1c), 7 point glucose day profiling (7PGM) 3 times weekly and continuous glucose monitoring (CGMS) for a minimum of 48 hours. A more detailed account of the study methodology and patients are described below.

Patient selection and exclusion criteria

Iron Therapy Group (A)

All patients selected for iron therapy had either absolute or functional iron deficiency as evidenced by serum ferritin values < 200 μg/L. All patients had haemoglobin ≤10.5 g/dL. Patients in this group were not on previous or concurrent ESA therapy and were vitamin B12 and folate replete. Intravenous iron was given as a single dose in the form of low molecular weight iron dextran (Cosmofer). This was delivered as an initial intravenous test dose of 100mg of iron over one hour followed by the remaining amount over 2-4 hours depending on the patient's body weight.

Erythropoietin stimulating agent (ESA) therapy Group (B)

All patients receiving ESA therapy had haemoglobin ≤10.5 g/dL and were considered iron, vitamin B12 and folate replete prior to initiation. Patients were considered iron replete following a serum ferritin value > 200 μg/L or having received intravenous iron at least 6 weeks prior to ESA therapy. ESA treatment was given in the form of darbepoetin alpha at 750 μg/kg fortnightly and continued throughout the period of the study. The dose of ESA was titrated monthly to achieve a target haemoglobin 10.5 – 12 g/dL.

Exclusion criterion:

Patients with known haemoglobinopathy, history of transfusion or bleeding with the last 6 months, previously treated with ESA, on renal replacement or with previous transplantation were excluded from the study.

Monitoring of glycaemic control

Patients in groups A and B were provided with the *Abbott Freestyle Freedom Lite* glucose sensor (Abbott Diagnostics, Maidenhead, UK). This particular device was selected due to its ease of usage and wide haematocrit (15-65%) range.

Patients received education regarding the correct technique, timing and method of obtaining finger prick tests upon agreeing to participate in the study. Patients were requested to perform 7 point glucose monitoring (7PGM) 3 times weekly one month before commencement of treatment until the end of the study. 7PGM was defined as pre-meal, 90 min post meal and pre-bed capillary glucose measurements. Results were from all measurements were then downloaded using the Co Pilot Health Management System (Abbott Diagnostics, Maidenhead, UK) and the results from the meter readings translated to Microsoft Excel and SPSS 16.0 where final data analysis were performed.

Continuous glucose monitoring (CGMS) was performed using the *Medtronic CGMS Ipro Continuous Glucose Recorder* (Medtronic Minimed, Northridge, US). Using this system, measurements of interstitial glucose levels were made 228 times over a 24 hour period. Result correlation was made using 7PGM results taken concurrently.

Patients were required to attend in the morning where the device was inserted by the thesis author. This device was left in place for a period of 48-72 hours. All patients had CGMS performed for at least 2 days prior to ESA therapy and once again at the end of the study. Results from the CGMS included at least a successful 24 hour profile over the monitoring period with no gaps > 120 mins.

The management of diabetes control were left to the patients and their health care professional. Treatment for glycaemic control was monitored throughout the study period.

Glycated haemoglobin:

All HbA1c samples were obtained with the patients fasting. Analyses of samples were made using ion-exchange chromatography via the Menarini HA-8160 HbA1c analyser (A. Menarini, Berkshire, United Kingdom).

All samples were stored at room temperature (22 $^{\circ}$C) and run within 24 hours from time of sample collection. It has been shown that there is no interference between carbamylated haemoglobin (present in uraemia) and HbA1c using this analyser (205). Patients in group A and B had samples taken at the one month before commencement of therapy and once again 4 months following treatment initiation.

Data analysis

All data was tabulated using Microsoft Excel and statistical analysis was made using SPSS 16.0 using paired t tests where appropriate.

Mean blood glucose (MBG) pre and post treatment was calculated by taking the average of the daily mean glucose values where there were 3 more capillary glucose readings per day. As glucose values were measured more frequently over CGMS monitoring periods, the results were weighted to ensure each measurement was proportional to the inverse of the total number of measurements taken the same day similar to that done in the A1c-Derived Average Glucose (ADAG) Study (61).

Power calculation:

Data from previous studies were used to calculate the statistical power required (56; 203). Assuming the intrasubject variation of HbA1c is Gaussian (209), 9 patients were required to detect a 1.2% fall in HbA1c in group A and 13 patients to detect a 1.0% fall in group B with 80% power to an alpha of $p<0.05$ using nQuery (Statistical Solutions Ltd, Cork, Ireland).

Ethical approval was obtained from the Local ethics committee prior to the commencement of the study. (LREC number 08/H1304/114)

3.1.3 Results

Patient data

Intravenous iron therapy (Group A)

Fifteen patients (9 M 6 F; all Caucasian, median age 72 years (IQR 68-74), median albumin to creatinine ratio (ACR) 6.3 (IQR 4.3-76.3)) agreed to participated in this arm of the study. Six patients were diet controlled and 9 patients were insulin requiring. The mean±SD follow up period was 16.4±3.7 weeks.

Erythropoietin Stimulating Agent Therapy (Group B)

Fifteen patients (11 M 4 F; all Caucasian, median age 70 years (IQR 62-75), median ACR 9.3 (IQR 6.0-93.4)) were recruited in this group. Four patients were diet controlled, 4 were on oral hypoglycaemic agents and 7 were insulin requiring. The mean follow up time in this group was 17.3±3.3 weeks. No patient received additional oral or intravenous iron therapy over the period of the study following the initiation of ESA treatment.

Glucose measurements and control

No new treatments affecting glycaemic control (e.g. oral hypoglycaemic agents, steroids, ß blockers) were initiated or altered over the study period in all patients.

The CGMS and the 7PGM data included ~ 1300 and 250 measurements per subject, respectively, for a total of ~ 1500 glucose tests over the entire study period. Using the 7PGM results there are a mean 4.7 readings a day of which 31% seven point profiles were complete. The median days of CGMS was 6. The results of the CGMS were retrospectively calibrated with the 7PGM readings performed over the similar period.

MBG in both groups did not change over the study period. The results of these are summarised in Table 8-9.

HbA1c values

Despite a lack of change of glycaemic control in the both groups, HbA1c concentrations fell significantly ($p<0.001$ and 0.013 respectively for Groups A and B). There was no linear relationship between the change in HbA1c and haemoglobin concentration values. (Group A, Pearson's 2 tailed,R^2=-0.329, p=0.23, Group B, R^2=-0.313, p= 0.25)

Subgroup analysis of Group B

In the group of patients receiving ESA therapy, there were 7 patients (5 M, 2F, median age 72 (IQR 62-79)) who received ESA therapy after iron treatment and 8 patients (6M,2F, median age 69 (IQR 61-74) who were received ESA only. All patients who also received iron were treated at least 6 weeks prior to ESA therapy initiation.

There appeared to be a non significant trend towards ESA leading to a further decrease in HbA1c following the initial fall due to iron (mean HbA1c 7.3% to 6.9%, p=0.36 following iron and 6.9% to 6.7%, p=0.13, following ESA). In contrast, the group of patients receiving ESA therapy without iron only had a significant fall in HbA1c from 7.3% to 6.5%, p =0.02.

MBG did not change in either group (9.12 vs. 9.21 mmol/L, p=0.47, ESA and iron vs. 8.21 vs. 8.26 mmol/L, p=0.71, ESA only) and there was a concurrent rise to haemoglobin (9.6 to 11.76 g/dL, $p<0.01$ vs. 9.4 to 11.3 g/dL, $p<0.01$) and haematocrit values (0.310 to 0.347, $p<0.01$ vs. 0.331 to 0.384) following therapy.

3.1.4 Discussion:

Erythropoietin stimulating agents and intravenous iron are commonly used therapies in the management of anaemia in patients with CKD. Patients with both DM and CKD have a higher prevalence of severe anaemia as compared to patients with CKD alone (99-101). Despite the increased usage of ESA agents, recent findings have shown that the correction of anaemia to levels of haemoglobin in excess of 12.5g/dL in patients with T2DM using this therapy has not led to an improvement in mortality but rather an increased risk of stroke. This needs to be interpreted carefully as the two groups received disproportionate amounts of IV iron. Indeed in the placebo group it was noted that there was in increase in the haemoglobin levels with ESA agents. Hence best practice would suggest that correction of functional and absolute iron deficiency should be obtained prior to commencement of ESA (119).

This is the first study to robustly show that iron and erythropoietin stimulating agent treatment result in a fall in HbA1c which is independent of glycaemic changes in patients with DM and CKD stage IIIb and IV.

Discordantly high HbA1c values as compared to glucose readings have been reported in previous studies and case reports on non DM patients with iron deficiency (206; 207). The correction of the iron deficiency in all these patient groups has lead to a fall in HbA1c values in these patients though the monitoring of glycaemic control of patients has not been as robust as compared to our study (using methods such as fasting plasma glucose or 2 pre meal readings a day).

It has been shown a fall in HbA1c concentrations following ESA treatment in patients with DM undergoing haemodialysis (205). Other than a single case report (204), there was scarce data to support the class effect of this therapy on patients not on haemodialysis.

Nakao et al. (205) reported a fall in HbA1c in non DM patients with CKD on haemodialysis following ESA therapy. The 1.2% fall in their study was much larger when compared to our results. A plausible explanation is that in contrast to our study,

iron therapy was given concurrently which has likely to have potentiated the HbA1c lowering effect reported. A proportion of patients in our study had both therapies and though a similar trend of combined lowering of HbA1c in this group, this failed to reach statistical significance.

Good glycaemic control in patients with DM and CKD has been shown to be associated with better survival rates (210). Proper assessment of glycaemic control is therefore vital if this is to be achieved. The results of our study show both statistically and clinically significant falls in the HbA1c following iron and ESA treatment (mean 0.4% following iron and 0.7% following ESA) in the absence of a change in glycaemic control.

From a practical view, the data from this study highlights several issues to which diabetes management can be improved in patients with DM and CKD. It shows that HbA1c can be unreliable and can fall following treatment with both iron and ESA therapy. It is essential that healthcare professionals are aware of the potential fluctuations of HbA1c that can occur in this patient group. Alternative methods for measuring glycaemic control such as capillary glucose testing and CGMS should be employed and therapy should not be based on the HbA1c value alone. This has particular significance when considering national, international or health service glycaemic targets such as the Quality and Outcome Framework (QoF) in the UK which almost exclusively use HbA1c as the sole index by which treatment success is judged.

Glycated albumin has been suggested as an alternative marker to represent glycaemic control as it was noted to be similar (in contrast to HbA1c which was higher) in patients with iron deficiency and pre ESA as compared to patients post therapy (211; 212). Though this may be true, further study is still required and better correlation between glycated albumin and glycaemic control is still needed to before this measurement to be more widely used.

The strengths of this study lies in the robust monitoring of glycaemic control in patients. 7PGM and CGMS were used in all patients and glycaemic control, treatment and HbA1c values were monitored closely. However, this study is limited by its relatively small numbers and though it managed to show that HbA1c values fell both with iron and ESA, there were insufficient numbers to confirm whether the combined effect of both therapies had an added HbA1c lowering effect as compared to a single agent given alone.

Intravenous iron and ESA are increasingly common therapies used in the management of anaemia in patients with CKD and DM. The present study has been able to confirm that reported changes in HbA1c following these treatments are indeed independent of changes in glycaemic control and so caution is warranted in the interpretation of HbA1c and management of glycaemia when based on this measurement alone. At a time when self-monitoring of blood glucose is being discouraged, especially in non-insulin treated patients (165), regular capillary glucose measurements, and the concurrent use of CGMS if available, seems essential in order to accurately assess glycaemic control in this group of patients.

Part 2:

Effect of intravenous iron and erythropoietin stimulating agent therapy on glycated albumin and glycaemic control.

3.2.1 Introduction:

As discussed in chapter 4 Part 1, glycated albumin (GA) has been proposed as a more reliable indicator of glycaemic control as opposed to glycated haemoglobin (HbA1c) in patients with iron deficiency and upon receiving erythropoietin stimulating agent (ESA) therapy (55; 211). However, the evidence on which these conclusions have been made in the absence of robust correlation with actual glucose values. Furthermore, majority of the studies evaluating this have been cross sectional in design and have been based on single GA readings per patient.

The present study was follow up from the study described in first part of this chapter. GA values were measured serially in the similar group of patients set to receive iron and ESA. The purpose of this was to study the effect of both therapies on GA values in the light of stable glycaemic control as evidenced by CGMS and 7PGM measurements.

3.2.2 Methods:

A more detailed description of the study design and patient characteristics has been described in the beginning of this chapter under *section 4.1.2*. Briefly, this was a prospective study of 30 patients with T2DM and CKD stage IIIB or IV (estimated glomerular filtration rate MDRD 15-44 ml/min/1.73 m^2) treated with intravenous iron and/or ESA between January 2009 and December 2009 inclusive.

Patients were divided into 2 groups. The first group (A) were patients who were to receive intravenous iron therapy and the other group (B) of patients were those requiring ESA treatment.

Samples were obtained a month leading up to treatment and once again 4 months into therapy. These samples comprised of glycated haemoglobin (HbA1c), glycated albumin (GA) and full blood profiles. Samples for GA were centrifuged at 3000 rpm for 15 minutes and then plasma was separated and frozen at -80 °C within 1 hour of sampling. All samples were analysed at the end of the study period. Serum GA was determined by enzymatic methods using albumin-specific protease, ketoamine oxidase and albumin assay (bromocresol purple) reagent (Lucica GA-L, Asahi Kasei Pharma, Tokyo, Japan). This analysis measures both GA and albumin concentrations. GA values were then calculated by dividing GA concentration by the albumin concentration and are expressed as percentages.

3.2.3 Results:

Glucose measurements and control

The CGMS and the 7PGM data included ~ 1300 and 250 measurements per subject, respectively, for a total of ~ 1500 glucose tests over the entire study period. Using the 7PGM results there are a mean 4.7 readings a day of which 31% seven point profiles were complete. The median days of CGMS were 6. MBG values did not change significantly over the study period.

Glycated albumin and HbA1c values:

Despite a lack of change of glycaemic control in the both groups, HbA1c concentrations fell significantly ($p<0.001$ and 0.013 respectively for Groups A and B) whilst GA values remained constant. The results of the study are summarised in Table 8-9.

3.2.4 Discussion:

Over the past few years, GA measurements have been proposed as an alternative marker to HbA1c in patients with iron deficiency and following ESA therapy (211-

213). Inaba et al. (211) and Peacock et al (213) both suggested GA values correlated better with chronic glucose levels as compared to HbA1c in DM patients undergoing haemodialysis following ESA therapy. A Japanese study by Koga et al. similarly found that GA levels were more stable in the presence of iron deficiency (212). However, a limitation to these studies lay in the fact that glycaemic control was not comprehensively represented using either CGMS or regular 3 weekly 7PGM measurements. Therefore, it remained possible that the changes in HbA1c values reported in these studies may in actual fact represent true glycaemic changes rather than a class effect from the offending drug and it was possible that glycated albumin values were not representative to these changes. Furthermore, GA values were only measured once in many of the trials. As GA levels represent glycaemic control for only 1-2 weeks preceding the assay, correlation between actual glycaemic changes and these measurements following iron and ESA therapy has not been conclusively made.

The present study comprehensively shows that glycated albumin values remain constant following iron and ESA therapy in parallel with glucose control. One of the study's strengths lie in the robust monitoring of glycaemic control in patients using 7PGM and CGMS with each patient having a mean 1500 glucose measurements made over the entire study period. Under these robust monitoring conditions, serial GA samples measured before and after the therapy remained unchanged as compared to HbA1c concentrations, confirming this measurement as the more stable marker. Calculation of mean blood glucose has been calculated in a similar fashion to the landmark ADAG study (61).

However, this study is limited by its relatively small numbers. Though the results did show that glycated albumin was more stable than HbA1c in patients receiving iron and ESA, the present study was unable to prove that changes in glycaemic control would be followed by a parallel shift in GA values in this patient group.

From a practical view, this study highlights several issues. The results reaffirm that glycated albumin values as the more reliable glycaemic marker than HbA1c in

patients receiving iron and ESA therapy. Though this may be the case, further studies are still required to confirm the target GA necessary to ensure a better prognosis and at which stage GA levels becomes the preferred alternative to HbA1c values. Furthermore, changes in glycaemic levels need to be correlated with GA values to ensure that these changes can be detected biochemically. Until these questions are answered, alternative measurement methods using regular capillary glucose measurements and CGMS in available, would appear essential to accurately assess the glycaemic control on these group of patients.

Table 8:
Comparison of HbA1c glycated albumin, Hb and glycaemic control in patients before and after iron therapy.

	Before iron mean (95%CI)	After iron Mean (95%CI)	p**
Glycated albumin (GA)	17.8 (15.1, 20.5)	17.7 (15.1,20.3)	0.59
GA/HbA1c ratio	2.48 (2.21,2.73)	2.62 (2.37,2.85)	<0.001
HbA1c (%)	7.40 (6.60,8.19)	6.96 (6.27,7.25)	<0.001
Hb (g/dL)	9.71 (9.32,10.05)	10.46(9.97,10.75)	0.001
Hct	0.302(0.285,0.316)	0.334(0.314,0.354)	0.007
Ferritin (μg/L)	122 (67,176)	307 (211,403)	<0.001
Mean blood glucose (mmol/L)	9.55 (8.20,10.90)	9.71 (8.29,11.13)	0.071
eGFR	34.0(31.9,36.2)	32.8 (30.4,35.2)	0.137

** paired t test

Table 9

Comparison of HbA1c, glycated albumin, Hb and glycaemic control in patients before and after ESA therapy.

	Before ESA mean (95%CI)	After ESA Mean (95%CI)	p**
Glycated albumin (GA)	16.7 (14.8,18.5)	16.9 (15.4,18.4)	0.71
GA/HbA1c ratio	2.33 (2.08,2.58)	2.60 (2.33,2.87)	0.004
HbA1c (%)	7.31 (6.42,8.54)	6.63 (6.03,7.36)	0.013
Hb (g/dL)	9.52 (9.18,9.86)	11.51(11.15,11.85)	<0.001
Hct	0.324(0.296,0.350)	0.378(0.341,0.398)	<0.001
Ferritin (μg/L)	344 (241,447)	332 (211,354)	0.37
Mean blood glucose (mmol/L)	8.72 (7.31,10.12)	8.78 (7.47,9.99)	0.893
eGFR	30.5 (28.6,33.4)	31.0 (27.3,33.8)	0.613

** paired t test

An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

Chapter 4:

Effect of bariatric surgery on glycaemic control, glycated haemoglobin (HbA1c) and glycated albumin (GA):

4.1 Introduction:

Patients with type 2 diabetes mellitus (T2DM) who undergo bariatric surgery have been shown to reduce their body mass index (BMI) by approximately 10-15 kg/m^2 and weight by 30-50 kg (215). Glycaemic control significantly improves within days following the procedure and a proportion of these patients have complete resolution of their diabetes (216).

Glycated albumin (GA) can be used as a marker of glycaemia over the 1-3 weeks prior to sampling, related to the fact that the half life of GA is approximately 19 days. It has been suggested it could be a more reliable assessment of glucose control than HbA1c in patients with conditions such as iron deficiency (217), haemodialysis (211) or following erythropoiesis stimulating agent therapy (See Chapter 4) .

Several previous studies have suggested that obese patients with T2DM have lower GA values as opposed to patients with lower BMI's. However, these studies have all been limited by them being cross sectional in design with some basing their findings on comparing GA with a single timed plasma glucose measurement and HbA1c (187; 188). Hence, it is not clear how significant changes in weight within the same individual might affect GA values or its utility as an indicator of glycaemic control.

This study sought to evaluate GA compared to HbA1c and mean glucose as an index of glycaemic control in patients with T2DM following bariatric surgery. The hypothesis was that the significant weight loss following this procedure would lead to a marked improvement in glycaemic control but that this same weight loss might make ensuing changes in GA be discordant with that of both HbA1c and mean glucose.

4.2 Methods:

This was a prospective study 9 month study of 13 patients with T2DM who had Roux-en-Y bariatric (RYB) surgery between Jan 2009 and Dec 2009 inclusive. Study patients had a BMI of at least 40 kg/m^2 and established T2DM as defined by the WHO criteria (218). All patients were attending a single outpatient service and the

decision for the procedure was made by a multi-disciplinary team with an expertise in bariatric surgery.

Patients were seen 3 months before RYB and at 2, 4 and 6 months after the procedure. At the initial visit, baseline bloods (HbA1c, GA) were sampled and clinical characteristics including body mass index (BMI), blood pressure (BP) and waist circumference measured. This was repeated again at every visit until the end of the study. Glycaemic control in this study was monitored using continuous glucose monitoring (CGMS) and 7 point daily glucose measurements (7PGM) over the entire study period.

Exclusion criterion:

All patients with known haemoglobinopathy, liver disease, hypothyroidism or chronic kidney disease were excluded from the study

Monitoring of glycaemic control and blood sampling:

The methods used to monitor glycaemic control and for blood sample analysis is similar to that described in Chapter 4A and Chapter 4B. Briefly, 7PGM in all patients was made using the *Abbott Freestyle Freedom Lite* glucose sensor (Abbott Diagnostics, Maidenhead, UK). Patients were requested to perform 7PGM starting at the initial baseline visit until the end of the study. Continuous glucose monitoring (CGMS) was performed using the *Medtronic CGMS Ipro Continuous Glucose Recorder* (Medtronic Minimed, Northridge, US). This was performed at every study visit and kept on for a minimum of 3 days. Results from the CGMS included at least a successful 24 hour profile over the monitoring period with no gaps > 120 mins. Mean blood glucose (MBG) was calculated in a manner similar to that done in the A1c-Derived Average Glucose (ADAG) Study (61).

Bloods was sampled for HbA1c and GA in the 3 months leading to surgery and once again at each study visit post procedure. All HbA1c and GA samples were drawn with the patients fasting.

Data analysis

All data was tabulated using Microsoft Excel and statistical analysis was made using *Analyse-it* (Analyse-it software Ltd, Leeds, United Kingdom). Paired t tests were used where appropriate.

Comparison of present study findings was made against a cross sectional data analysis correlating GA and HbA1c values. This data was based on 268 randomly selected blood samples from patients with DM performed by the local laboratory service. The correlation of this is seen in Figure 3.

4.3 Results:

There were a total of 13 obese T2DM patients were recruited for the present study (6F 7M, median age 52 (IQR 44-64)). The baseline characteristics of the patients pre and 6 months post surgery is detailed in Table 10. At the end of the study post RYB surgery there was a significant fall in weight, BMI, waist circumference and glycaemic indices.

Mean blood glucose measurements:

The CGMS and the 7PGM data included ~ 1400 and 200 measurements per subject, respectively, for a total of ~ 1500 glucose tests over the entire study period. Using the 7PGM results there are a mean 3.9 readings a day of which 24% seven point profiles were complete. The median days of CGMS was 7. The results of the CGMS were retrospectively calibrated with the 7PGM readings performed over the similar period.

Changes in glycaemic indices:

All indices of glycaemia improved following RYB surgery. The summary of the indices and the percentage fall of the each index from baseline at each visit is summarised in Table 11. At the end of the study period, the percentage change of GA and HbA1c from baseline was virtually identical, both being lower than the percentage change in MBG.

In the present study, the mean HbA1c value fell by 3.1% and GA by 6.2% after 6 months RYB surgery. When the similar change in HbA1c was applied to larger diabetes population data collected (Figure 3), the anticipated fall in GA would have been 6.6%. Therefore, GA values in the bariatric patients did not appear to fall by an amount that was very different to what would have been expected from the HbA1c change despite very marked reductions in BMI (51.5 vs. 36.3 kg/m2) in patients pre and post surgery.

4.4 Discussion:

This is the first known study that has attempted to correlate changes in BMI and GA values within the same patients. This was done by prospectively following up a group of subjects with DM undergoing bariatric surgery. However, the present study failed to show any definite correlation between BMI and GA beyond that expected from their fall in HbA1c and mean glucose.

The strengths of this study were in its prospective design, the serial measurements of GA and HbA1c and the robust monitoring of glycaemic control. The main weakness of the study lies in the small number of study patients. Therefore the inconclusive findings reported in this study could be either as the result of a lack of statistical power (i.e. a type 2 error) or the true absence of any relation between GA and BMI.

Previous studies have proposed that obese patients with T2DM have lower GA values than lean ones (187; 188). This was first reported by Koga and colleagues when they proposed negative correlation between BMI and GA ($R=0.25$, $p<0.001$) when they analysed the results from 426 Japanese patients with both type 1 and type 2 DM

(187). This relationship was not seen when HbA1c or plasma glucose values were compared. Miyashita et al. reported that obese patients had significantly lower GA (2.7±0.3 vs. 3.0±0.4%) and absolute glycated albumin (aGA) (0.8±0.2 vs. 0.9±0.2 g/dL) values for the same HbA1c (188). The authors of both studies were unable to conclusively account for the underlying mechanism behind these findings.

It must be highlighted that the present study holds several distinct differences when compared to these previous studies. Firstly, past publications have been based on cross sectional data and GA values in these studies only measured once in each individual. Therefore, any actual changes BMI have never been correlated with GA values. Secondly, glycaemic control has been limited to single timed plasma glucose measurements and HbA1c as opposed to the more robust monitoring methods used in the present study In other words, the relationship reported between glycaemic control and GA values may have been influenced by acute changes in glycaemic control in the short time period leading on to these previous study. Furthermore, 'obese' patients recruited to a previous study only had have a BMI>25kg/m^2 (mean BMI was 28.2kg/m^2) compared to the average of 51.5kg/m^2 initially in this study.

Though the final percentage changes to glycaemic indices at the end of the study were nearly identical, the fall in HbA1c and MBG values appeared to lag behind GA. This finding could be explained by the relatively higher glucose values in the first few weeks following surgery which would have influenced both HbA1c and MBG more than the shorter-term assessment of GA.

In being unable to confirm cross sectional studies which showed an inverse relationship between GA and BMI, the present study highlights an important clinical issue. Previous study results have cast doubt in using GA as a marker of glycaemic control since numerous pharmacological agents (both new and old) for the treatment of DM can exert a positive or negative effect on the body weight. The present study results found here in patients who showed massive weight change (averaging nearly 30%) raises doubt around the validity of that concern and therefore warrants further investigation.

GA has been suggested as a better indicator to glycaemic control to HbA1c in several physiological and pathological conditions. This study has shown it may also be a better marker than anticipated in T2DM patients undergoing bariatric surgery. It could therefore be included still be used with HbA1c, regular capillary glucose measurements and CGMS as a marker of glycaemic control in this group of patients.

Table 10

	Pre bariatric surgery	Post bariatric surgery	p
Waist circumference (cm)	128.4 ± 12.7	105.5 ± 11.7	<0.001*
Weight (kg)	137.6 ± 24.7	97.2 ± 17.5	< 0.001*
BMI (kg/m2)	51.5 ± 6.3	36.3 ± 5.4	< 0.001*
HbA1c (%)	9.2 ± 2.1	6.1 ± 0.8	0.001*
MBG (g/dL)	12.1 ± 3.7	7.5 ± 2.1	0.001*
aGA (g/dL)	0.8 ± 0.2	0.45 ± 0.1	0.002*
GA (%)	18.5 ± 5.1	12.3 ± 3.2	0.003*

Table 10: Patient characteristics and glycaemic markers pre and 6 months post surgery. Values are expressed as mean ± standard deviation (SD).

aGA: absolute GA values.

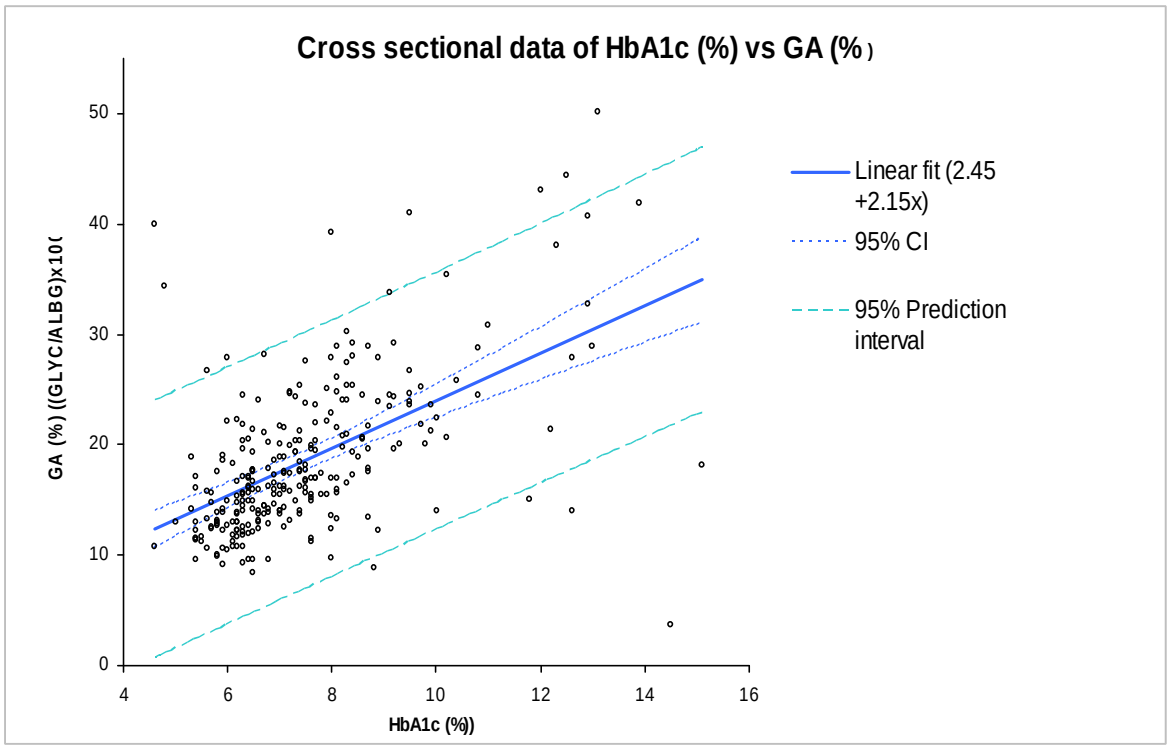
* paired t tests

Table 11:

	Pre surgery	Post surgery Visit 1	Post surgery Visit 2	Post surgery Visit 3
HbA1c (%)	9.2 ± 2.1 (reference)	7.2 ± 1.8 (21.5%)	6.5 ± 1.3 (28.9%)	6.2 ± 0.8 (32.9%)
MBG (g/dL)	12.1 ± 3.7 (reference)	8.6 ± 3.1 (29.6%)	7.6 ± 2.7 (37.2%)	7.5 ± 2.1 (38.2%)
GA (%)	18.5 ± 5.1 (reference)	12.3 ± 4.7 (33.3%)	11.9 ± 4.0 (35.8%)	12.3 ± 3.2 (33.4%)

Table 11: Glycaemic indices pre surgery and at each following study visit. Values are expressed as mean ± SD. The brackets at each show the percentage change from baseline.

Figure 3: Cross sectional population data of HbA1c (%) and GA (%)



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An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

Chapter 5:

Evaluating the use of plasma glucose and HbA1c measurements in the diagnosis and identification of patients with risk of diabetes mellitus

Chapter Introduction:

Both fasting and post load challenge plasma glucose measurements have been universally accepted as the method used to diagnose DM for more than 30 years (8). Following the International Expert Committee consensus in 2009, glycated haemoglobin (HbA1c) was proposed as a potential alternative or replacement diagnostic measurement (see *section 1.3.2 Issues surrounding HbA1c in the diagnosis of diabetes mellitus*). The next 2 parts in this chapter scrutinise the utility of each of these measurements (plasma glucose and HbA1c) in clinical practice.

Part 1:

Impaired fasting glucose and impaired glucose tolerance: follow-up rates over 2 years within a primary care setting

5.1.1 Introduction:

The presence of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) is associated with a subsequent increased risk of developing Type 2 diabetes mellitus (T2DM) and an increased mortality from future cardiovascular disease.(19; 219) It is therefore essential that these two conditions are monitored regularly with lifestyle advice and intervention provided where necessary.

Guidelines advocate that patients with IFG and IGT are screened at least annually with either an oral 75g glucose tolerance test (OGTT) or fasting plasma glucose.[2] To this effect, we followed up all patients who were diagnosed in 2006 with IFG, IGT or both until 31st December 2008 within primary care to ascertain whether this group was being appropriately retested.

5.1.2 Methods:

All patients undergoing a 75g oral glucose tolerance test (OGTT) in primary care in the NHS Hull and East Yorkshire PCT in the year 2006 were included in the analysis. Data from this study was obtained from the laboratory serving the whole population. Patients were followed up until 31st Dec 2008.

Approval was obtained from the Trust Audit Department prior to the commencement. Publication of this data was also authorised by the Caldicott guardian. The criterion for diagnosis of IFG, IGT and T2DM was based on the WHO thresholds (220). All patients who were screened for gestational diabetes or were deceased over this period were excluded from the analysis.

5.1.3 Results:

There were a total of 2495 patients during 2006 who underwent an OGTT. Out of this number, there were 1446 patients with abnormal glucose results. (445 had IFG, 289 IGT and 329 patients with both). There were 383 patients diagnosed with T2DM.

Of the 1063 patients with IFG/IGT, (median (IQR) age 67 (57 to 76) years, 625F, 438M), 838 (80.9%) patients had at least one further glucose test from the index test until 31st December 2008 after a median (SD) of 13.5 (6.9) months.

FPG was repeated at least once in all 838 patients, out of which 423 of this number had a repeat 2 hour OGTT. From these tests, 256 patients had persistent IFG, 160 patients had IGT and 137 patients had either a FPG glucose greater than 6.9 mmol/L or a 2 hour glucose level of 11.0 mmol/L or more.

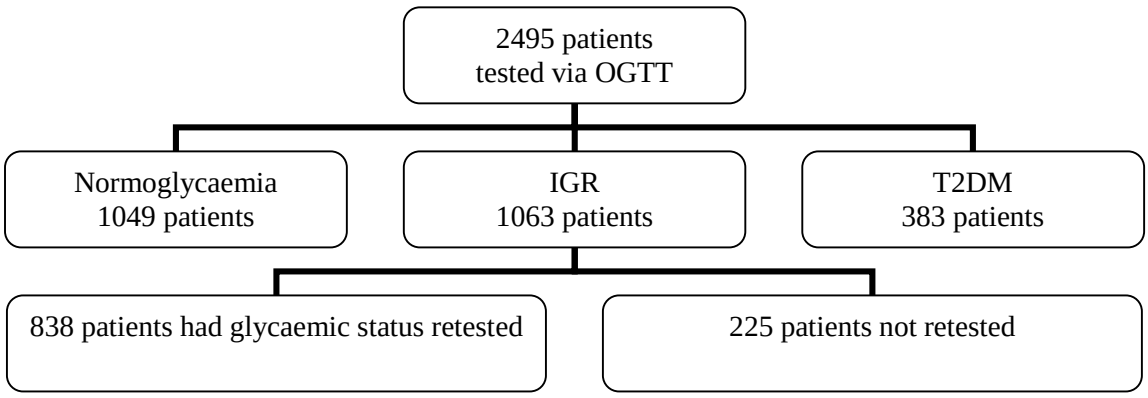
Crucially this meant that 225 (21.1%) of patients (median (IQR) age 62(48 to 72) years, 127F, 98 M) did not have a repeat test over the median 30.5 months they were followed up. HbA1c was measured in 23 of these patients without the presence of a repeat FPG or OGTT. Conversely, 28 (2.6%) patients with a biochemical diagnosis of T2DM (11 patients with 2 hour post OGTT of 11.0 mmol/L of more and 17 patients who had a FPG greater or equal to 7.0 mmol/L on 2 or more occasions) did not have follow up HbA1c measured more than 9 months after the abnormal test result.

5.1.4 Discussion:

Our data has shown that approximately 1 in 5 patients with established IFG and IGT did not undergo appropriate repeat testing after a median of 30.5 months following initial identification in the community. Although the analysis is likely to be limited by its retrospective nature and the fact that subjects may have moved out of the area in the interim, the 30 month follow-up period could not possibly explain the percentage of patients not being retested.

In summary, our findings highlight the need for a systematic means of following up patients identified with IFG/IGT in the community, especially as more routine diabetes care is devolved to primary care.

Figure 4: Flowchart of the proportion of patients with impaired glucose tolerance (IGR) followed up over 30 months



Chapter 5
Part 2

New Recommendations in Diagnosis of Diabetes Mellitus from the Department of Health: Comparing the old and new.

5.2.1 Introduction:

Recent guidelines from the UK Department of Health (DoH) recommend a choice of diabetes screening algorithms as part of their 'NHS Health Check'. This comprises either the traditional use of fasting plasma glucose (FPG) cascading on to a 75g oral glucose tolerance test (OGTT) if impaired fasting glucose (IFG) is present or, alternatively, HbA1c measurement cascading onto an OGTT if the HbA1c is 6.0-6.4%. Patients could be diagnosed as having diabetes without a cascade if symptomatic and following either a single FPG confirmed to be ≥7mmol/L or HbA1c ≥6.5%.(18) By definition, therefore, a single measurement of HbA1c of less than 6% in an individual leads to a recommendation that no further testing of glycaemic status is necessary.

5.2.2 Methods:

We have compared the 2 different approaches amongst patients who have had both OGTTs and HbA1c requested in the community.

All patients who had a 2 hour plasma glucose (2hPG) value greater than 11 mmol/L on OGTT and happened to have a corresponding HbA1c were included in the analysis. Patients were included in the study period extending from 1st Jan 2007 to 31st Dec 2008. Patients with gestational diabetes and who were deceased over this period were excluded from the analysis.

Data was obtained from the laboratory serving the whole Hull and East Yorkshire population. Audit approval was obtained prior to commencement of this analysis.

There were a total of 1135 patients who had a positive 2hPG OGTT and a HbA1c over this period.

5.2.3 Results:

Of this number, 272 patients (median (IQR) age 67 (58 to 75), 135 F, 137 M) had a corresponding HbA1c done within 2 weeks of the index OGTT. All OGTTs were preceded by an abnormal FPG. Median HbA1c of this group at the initial diagnosis of T2DM was 6.4% (IQR 6.1-7%).

Of the 272 patients, 218 (80.1%) of patients with a 2hPG greater than 11 mmol/L had HbA1c values greater or equal to 6%. Crucially, this means that 54 (19.9%) patients who were screened positive using the 2hPG on OGTT had a HbA1c value of less than 6%. These results are summarised in a flow chart in Figure 3.

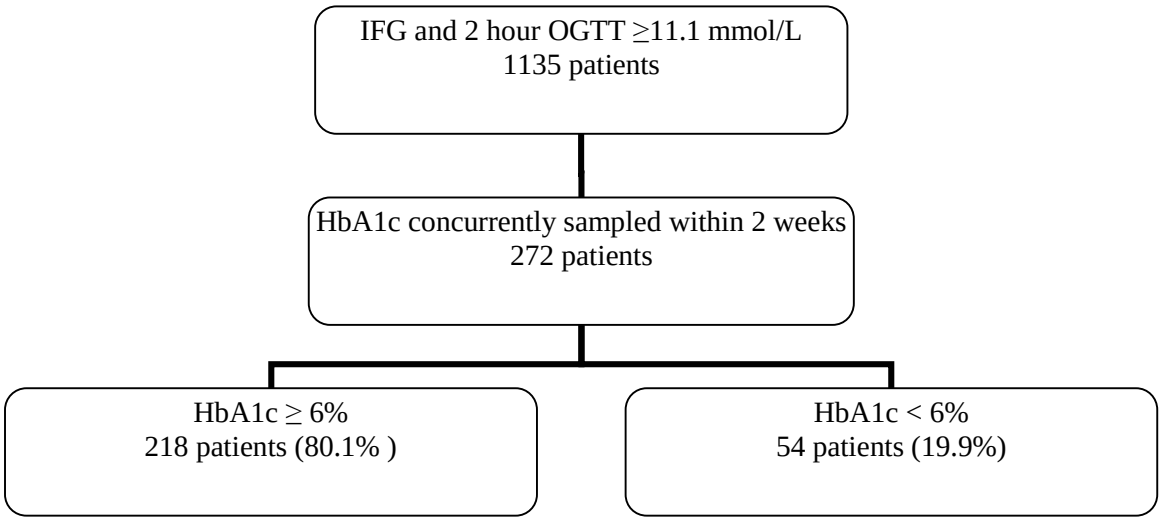
5.2.4 Discussion:

Recent DoH guidelines advocate the use of a single HbA1c reading as an option when screening for T2DM in patients at risk of developing the disease. Our analysis shows that almost a fifth of these patients who would normally have been diagnosed with T2DM using the usual glucose criteria option would not have had any further follow up for up to 2 years if HbA1c was used as the only index test.

Our analysis is inherently limited by its retrospective nature, but it is consistent with previous studies showing that HbA1c seems to identify a different group of patients to FPG or 2hPG. Indeed, even when just using FPG criteria it has been noted that up to a third of patients with a FPG≥7mmol/L can have a HbA1c <6%.(220)

In summary, choosing the HbA1c based algorithm in the NHS Health Check document will reduce the proportion of patients identified with diabetes and may delay the diagnosis of patients when compared to the solely glucose-based algorithm. This should be considered when deciding which testing pathway to implement.

Figure 5: Flowchart OGTT results and corresponding HbA1c values.



An evaluation of factors that affect glycaemic control and its measurements in diabetes mellitus

Chapter 6:

Summary conclusion

This thesis evaluates a number of issues surrounding several factors that affect glycaemic control and its measurements in patients with DM. Glycated haemoglobin (HbA1c) is given particular focus but other alternate glycaemic indices including glycated albumin (GA), self monitoring of blood glucose using capillary glucose measurements and CGMS are also evaluated.

Environmental factors have been known to have an adverse effect on health and the management of chronic illness. Patients who have sedentary lifestyles are known to be at increased risk of developing DM in the future (147). A poor diet and significant psychological stress can result in deterioration in glycaemic control (151; 194). The first chapter of the thesis summarises a longitudinal study of the effect a flooding disaster had on patients with DM. Using HbA1c as the method of glycaemic assessment, this study demonstrated a significant deterioration in diabetes control in patients affected by the flooding disaster. The effect was particularly evident in patients who were insulin treated as compared to those on lifestyle or oral therapy. This present study shows that the proceeding months following a natural disaster can affect a population with DM by causing significant disruptions to their glycaemic control.

This study brings to light several other unanswered questions which need to be explored by future studies. Firstly, studies on the effect of a natural disaster on patients with diabetes mellitus are scarce. The data from this present study adds to the small pool of knowledge available as it identifies insulin using patients with DM as the more vulnerable group over periods of natural disaster. Future studies need to focus on methods to better prepare this group of patients to cope over such periods and also the long term effects of such disasters to health outcomes in patients with DM.

In order to appropriately treat or manage patients with DM, it is crucial that an accurate assessment of glycaemic control is made. At the present time, HbA1c is the universal marker used in clinical practice. HbA1c is dependent on biochemical

changes that occur between glucose and the red cell and therefore, any changes in glycaemia or the erythrocyte can potentially influence its value. The next study in the thesis shows that both intravenous iron and erythropoietin stimulating agents in DM patients with CKD can result in a fall in HbA1c values which is independent of any true change in glycaemic control. Therefore, caution is required in the interpretation of HbA1c in this patient group and therapy should be tailored based on other measurements such as capillary glucose and CGMS. Furthermore, the present study shows that glycated albumin (GA) measurements appear to be a better indicator of glycaemic control than HbA1c in these patients because its values appear to be unaffected by either iron or ESA therapy.

There is significant scope for further study particularly in further establishing the combined class effect of both therapies on HbA1c and also in glycated albumin measurements. A study of a longer follow up duration may help to determine if the HbA1c fall noted is sustained or if further changes are expected. The present study showed a non significant trend towards a combined HbA1c lowering effect when both iron and ESA were given in succession to patients. Unfortunately, the study was not powered to show this change. This needs to be explored further using a larger patient sample in future studies. In parallel to this, more research is needed to ascertain whether the degree of erythropoietin or iron deficiency has any bearing on the degree of disconcordance seen within the HbA1c values.

To better define the factors that influence glycated albumin (GA) values, the next study attempted to evaluate the correlation between GA and BMI in a group of patients undergoing bariatric surgery. Previous evidence has suggested a negative correlation between the 2 indices. However, these results were based on cross sectional data and the determination of glycaemic control have been based on a single timed plasma glucose readings and HbA1c (187; 188). The study in the thesis attempted to re-examine the correlation between GA and BMI but with more robust monitoring of glycaemic control and being prospective in study design. Despite significant difference in weight pre and post surgery, the study was unable to show

any definite correlation between BMI and GA beyond that expected from their fall in HbA1c and mean glucose.

These results are in contrast to findings previously published. It suggests that GA values may be less susceptible to this problem than previously suggested and may still be useful as an indicator of glycaemic control in patients in spite of significant weight changes. This study opens several other avenues for further research. A larger prospective study on non DM patients undergoing bariatric surgery may better discern the relationship between BMI and GA. Furthermore, most of the studies on GA have been performed on patients of Japanese descent and the definition for obesity for the studies has been defined as a BMI > 25 kg/m^2 (187; 188). This is clearly different from the Western population and more studies on a predominantly Caucasian population will be required if the GA is to accepted as be a clinical glycaemic marker.

Both the studies in renal patients and those undergoing bariatric surgery lay the foundation for further study on GA measurements. Though in some conditions, GA values may be a better indicator of glycaemic control, there is scope for future studies to determine the target GA necessary to ensure a better prognosis and at which stage GA levels becomes the preferred alternative to HbA1c values. The influences of other indices such as uric acid levels, triglycerides and BMI on GA and the mechanism in which its values are affected need close scrutiny. Furthermore, changes in glycaemic levels need to be correlated with GA values to ensure that these changes can be detected biochemically.

Rising glycaemic levels even below the thresholds of DM have been associated with an increased risk of mortality and morbidity (122). A significant proportion of patients shown to have DM have had the condition for many years before the diagnosis was made (12). Consequently, there is a need for an effective method of diagnosing and screening of patients who are at risk of developing DM. National guidelines from bodies such as the Department of Health and the Joint British Society have produced recommendations on this issue in an effort to ensure that there is timely intervention in the event of a diagnosis of hyperglycaemia. It was shown that

measuring plasma glucose (as a timed/challenged sample) or HbA1c alone resulted in a significant proportion of patients who were either not diagnosed or inadequately followed up.

The barriers preventing the follow up of these patients are not fully known but the need for repeat fasting specimens for glucose may be one of them. Though the HbA1c may result in a number of patients not being diagnosed with DM as compared to using plasma glucose, the test is much easier to perform as the sample is more stable and does not require patients to fast (10). Therefore, further study is required after the implementation of these recommendations on both screening and diagnostic rates. The suggestion of setting glycaemic thresholds incorporating both measurements on a national/international level (220), may be a solution to this problem and enable a more accurate method of diagnosing and following up patients at risk of DM.

In conclusion, the ability to accurately assess glycaemic control provides vital information that can be beneficial in the management of patients with diabetes mellitus. This thesis successfully addresses some disputes surrounding the different indices to glycaemic control and the factors that can affect glucose levels. Knowledge of the suitability of each of these measurements and when to anticipate potential glucose fluctuations can aid the healthcare provider in deciding the most appropriate therapy to match the clinical scenario. Although numerous methods of glycaemic assessment exist, none of them are universally applicable to every patient and so should be used as a complete replacement for clinical judgement.

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